STRUCTURAL REQUIREMENTS OF MICROSOMAL *N*-OXYGENATIONS DERIVED FROM STUDIES ON AMIDINES

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CONTENTS

SUMMARY

A short description of the chemical and pharmacological properties of amidines is followed by a comprehensive discussion of investigations on the *N*-oxidative biotransformations of amidines. The results of these investigations have confirmed the author's hypothesis, based on mechanisms, that *N*-oxygenation by the cytochrome P-450 enzyme system is observed particularly when N-dealkylation is not possible because of the absence of hydrogen atoms on the carbon atoms adjacent to the nitrogen atom, α-H-atoms. The results obtained with amidines are discussed in their relationship to other microsomal *N*-oxygenations both by cytochrome P-450 and flavine-containing monooxygenase. Attempts are made to deduce a scheme for predicting *N*-oxygenations.

I. INTRODUCTION

Practically all organic drugs (91% of 1000 active principles /1/) and numerous environmental chemicals contain nitrogen in the form of widely differing functional groups. As is the case with carbon, oxidative rather than reductive processes at nitrogen are the predominant metabolic reactions /2,3/. Following the pioneering work of Kiese /4/ on aromatic amines, the biotransformations of, in particular, weakly basic nitrogen-containing functions were investigated intensively and new biotransformation routes were discovered /5/.

Since *N*-hydroxylated derivatives are intermediates in the formation of reactive and toxic compounds /2,5,6/, investigations on *N*-oxygenation processes are of particular interest.

Principally, two microsomal monooxygenases have to be taken into consideration for *N*-oxygenations: the cytochrome P-450 enzyme system and the NADPH-dependent, flavine-containing monooxygenases /5/. The latter was purified for the first time by Ziegler /7/. Numerous attempts have been made to define the properties of nitrogen in the widely differing functional groups which determine whether oxygenation by one or the other of the monooxygenases will occur. For example, Gorrod /8/ suggested that pK_a a values, hybridisation, or positions of the tautomeric equilibrium could be responsible factors.

Others /9, 10/ inlcuding ourselves /11/ have pointed out that the

catalytic mechanisms play a decisive role as to whether or not an oxygenation at a specific nitrogen-containing structural element can take place. Thus, based on investigations with amidines, we postulated that an *N*-oxygenation by the cytochrome P-450 enzyme system can take place especially when a dealkylation is not possible because of the absence of hydrogen atoms on the carbon atoms directly adjacent to the nitrogen atom, α-H-atoms /11/. In this short review, experimental investigations on amidines which support this conclusion will be discussed. Since amidines do not appear to be substrates for the flavine-containing monooxygenase /12/, the structure-activity relationships are based on cytochrome P-450. It would seem appropriate to make separate predictions for microsomal *N*-oxygenations by the two monooxygenases since they exhibit different mechanisms /10/.

In addition to the presentation of the structural prerequisites for the *N*-hydroxylation of amidines, the chemical, pharmacological and toxicological properties of amidines and their *N*-oxygenated derivatives will be mentioned briefly.

II. PROPERTIES OF AMIDINES

2.1 Chemical Properties

The present article is concerned only with typically basic amidines which possess high pK_a values as a consequence of forming highly mesomerically stabilised cations; for example, the pK_a value of benzamidine (Figure 1) is 11.6 /13/. Thus, amidines are even more basic than aliphatic secondary amines, the pK_a values of which in water are at the most just over 11 /14/. This is, of course, only valid for amidines in which none of the nitrogen components form part of an aromatic system or which do not have a strongly electron-accepting substituent on the nitrogen atom.

It is thus interesting that amidines undergo biotransformations in spite of the fact that, under physiological conditions, they exist almost exclusively in the protonated form, which generally has high aqueous solubility /12/.

Under physiological conditions benzamidines are stable towards hydrolysis; formation of the corresponding amide could not be observed in any of our biotransformation experiments.

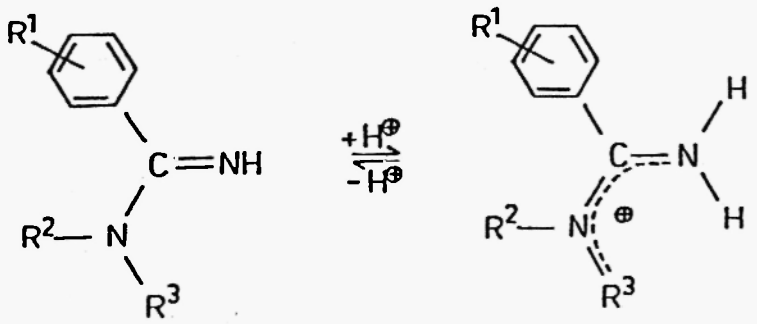


Fig. 1: Protonation of benzamidines 1.

Amidines are formally derived from carboxylic acids by substitution of the two oxygen atoms for nitrogen atoms. Thus, they possess the same oxidation level as carboxylic acids and their derivatives. Amidines should not be confused with imines (oxidation state of aldehydes and ketones) or guanidines (oxidation state of carbonic acid derivatives) although this is done occasionally, for example, amidines have erroneously been described as a class of imines /15/. This is wrong not only for purely formal reasons but also because of the differing chemical properties and differing behaviour in biotransformation reactions (see below). Analogously, the *N*-oxygenated derivatives (oximes from imines, amidoximes from amidines, *N*-hydroxyguanidines from guanidines) formed by *N*-hydroxylation have very little in common.

With regard to chemical properties, amidines resemble guanidines and aliphatic amines which are also strongly basic and stable towards hydrolysis /11/. In contrast, imines are not strongly basic and compounds lacking an N-substituent undergo hydrolysis to ketones or aldehydes, at a rate dependent on the substituents at the carbon atoms /16/.

2.2 Pharmacological Properties

Amidine functions are components of numerous active compounds /17/. In this respect, the aromatic diamidines in particular should be mentioned; these compounds were developed mainly because of their

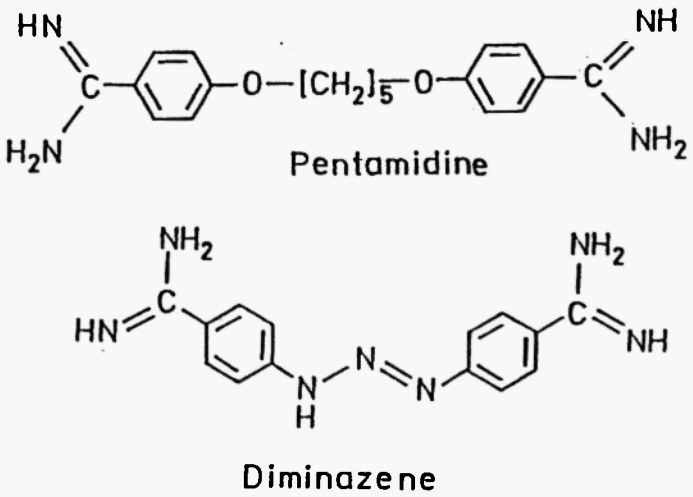


Fig. 2: Diamidines with trypanocidal activities.

activity towards trypanosomes /18/. The most important representatives of this group are pentamidine and diminazene /18/ (Figure 2). Pentamidine is one of the drugs of choice for the treatment of pneumonia caused by *Pneumocystis carinii*, a very frequently occurring secondary infection in AIDS patients /19/.

These diamidines also exhibit other interesting properties: they are strongly bonded to DNA /20/, some of them can inhibit tumour growth /20/, and, furthermore, benzamidines are excellent thrombin inhibitors /21/.

Amidine functional groups are also found in other drugs of widely different therapeutic classes /17/ such as chlordiazepoxide and the insecticide chlorophenamidine. Some amidines moreover have pronounced antihypertensive properties, for example the amidine analogue of guanethidine /22/. At the same time, these properties represent one of the most serious side-effects observed with the aromatic diamidines /20/.

Since several groups of drugs possess amidine functional groups bearing an aromatic moiety bonded to carbon, benzamidines were selected as the model substances for our biotransformation experiments.

III. STUDIES ON THE METABOLISM OF AMIDINES

Before metabolic studies were performed, the potential metabolites were chemically synthesised for comparison purposes; in some cases structures were confirmed with the aid of ^{15}N-NMR. Compound stability and detection conditions were checked using these synthetic reference standards. This was done in order to ensure that if formed the *N*-oxygenated biotransformation products could be detected with certainty and that unambiguous conclusions could be drawn.

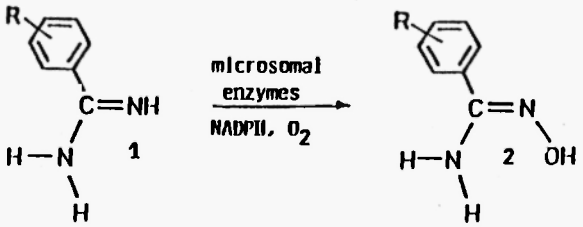
Biotransformation experiments were performed *in vitro* using the 9000g or 12000g supernatants, or the microsomal fraction of rabbit liver homogenates as enzyme sources.

Qualitative detection of the metabolites was achieved mainly by thin layer chromatography (TLC) and, after elution from the plates, by mass spectrometry (MS). Data obtained were compared with those of the synthetic reference substances. Quantitative studies were performed using newly developed high-performance liquid chromatographic methods (HPLC analysis). Full experimental details can be found in references /12,23,24/.

3.1 N-Oxygenations of Unsubstituted Benzamidines

After the first qualitative detection of the microsomal *N*-hydroxylation of *N,N*-unsubstitued benzamidines 1 to the benzamidoximes 2 (see Equation (1)) had been achieved /12/, this novel biotransformation route was quantitatively analysed in detail /11, 23/.

(1)



The characteristics of the microsomal *N*-hydroxylation of the benzamidine 1 (R = H) to the benzamidoxime 2 are summarized in Table 1 /23/. Since involvement of the flavine-containing monooxygenase had been excluded by preliminary experiments using the purified

TABLE 1

Effect of cofactors and of inhibitors of cytochrone P-450 on the N-oxygenation of benzamidine, and the N-demethylation of N-methylbenzamidine by rabbit liver microsomal enzymes.

Enzyme	Incubation mixture	nmol benzamid oxime/min per mg protein	nmol benzamid-ine.HCl/min per mg protein
9000 g	complete	0.33 ± 0.13	0.45 ± 0.17
Supernatant	– NADPH	< 0.01	< 0.033
	– O_2	< 0.01	0.12 ± 0.07
	– Mg^{2+}	0.20 ± 0.06	0.31 ± 0.13
	+ SKF 525A (5mM)	< 0.01	< 0.033
	+ KCN (25 μM)	0.21 ± 0.11	0.38 ± 0.14
	+ Metyrapone (0.1mM)	0.23 ± 0.09	0.16 ± 0.06
Microsomes	complete	0.27 ± 0.17	0.40 ± 0.11

Values are presented as the means of determinations from three animals ± S.D. For experimental details see /23, 24/.

enzyme /12/, the observed data, especially the inhibition by carbon monoxide (see Table 2), are consistent with metabolism by the cytochrome P-450 enzyme system /23/.

Unsubstituted and *N*-monosubstituted benzamidines have been shown to exist exclusively in the (*Z*)- rather than the (*E*)-configuration /25/, by ^{15}N-nuclear magnetic resonance spectral investigations /26/. Presumably, formation of intramolecular hydrogen bridging bonds contributes to the stability of these (*Z*)-isomers. Hence, it was not possible to study the stereoselective formation of amidoximes from amidines.

For ring-substituted benzamidines, a significant correlation was found between the logarithm of the maximum rates (log $V_{max}X$) and the Hammett σ_p constants (N = 6; correlation coefficient r = 0.984) which confirms the validity of the Hammett or the free enthalpy relationship for the *N*-oxygenation of *para*-substituted benzamidines (see Figure 3) /27/. From the slope of the line in Figure 3, a reaction

TABLE 2

Effect of various CO/O_2 concentrations on the rabbit liver microsomal enzymic oxygenation of benzamidine to benzamidoxime and N-demethylation of N-methylbenzamidine to benzamidine.

CO/O_2	% CO	% O_2	% N_2	N-oxygenation of benzamidine*	N-demethylation of N-methylbenzamidine*
Control	0	5	95	100	100
1	5	5	90	71	78
2	10	5	85	57	60
3	15	5	80	50	48
5	25	5	70	45	27
8	40	5	55	30	19

* Results expressed as a % of control enzymic activity.

Values are presented as the means of three determinations (three separate samples of one homogenate); liver preparations from two other aninals gave similar results. For experimental details see /23, 24/.

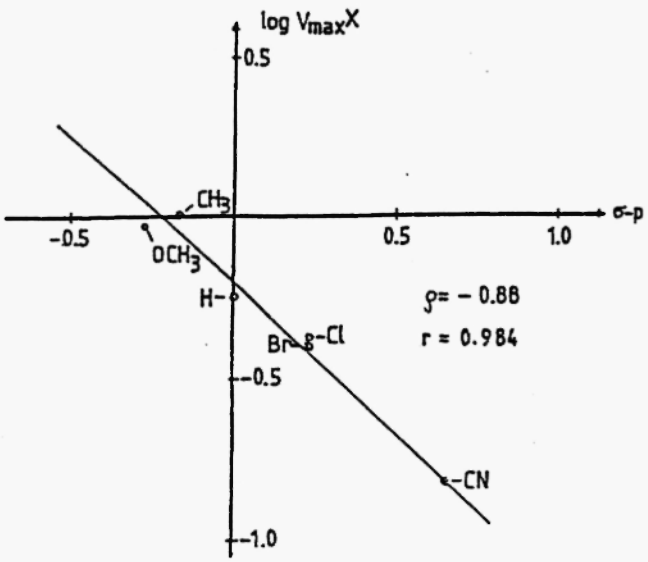
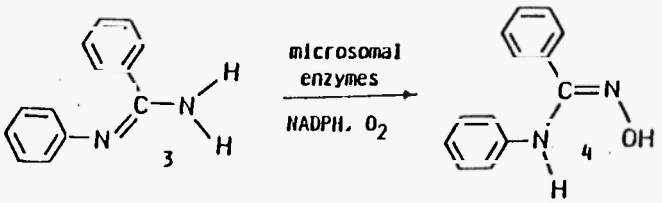


Fig. 3: Correlations of log vmaxx with σ-*para* for the N-oxygenations of *para*-substituted benzamidines by the 12,000g supernatant fractions of rabbit liver homogenates /27/.

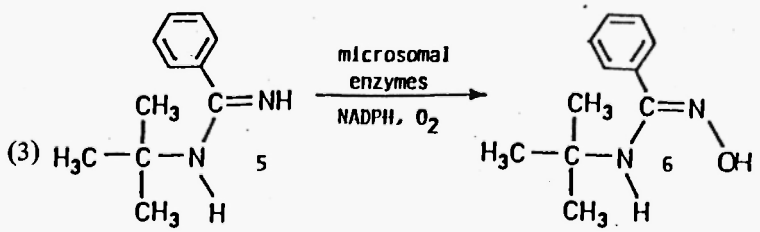
constant of $\rho = -0.88$ was obtained. The negative sign signifies that the presence of electron-donating groups facilitates the attack of the enzyme on the amidine system whereas the presence of electron-acceptors hinders it /27/.

3.2 N-Oxygenations of N-Substituted Benzamidines

In the series of benzamidines bearing aromatic substituents at nitrogen, the *N*-oxygenation of *N*-phenylbenzamidine 3 to the corresponding benzamidoxime 4 was confirmed (Equation (2)) /28/. Compound 3 exists predominantly in the tautomeric form shown in which the double bond is conjugated with the aromatic system, whereas the amidoxime structure in the (*Z*)-configuration is favoured in the *N*-hydroxylated product 4 /26/.

(2) 

In the series of *N*-alkyl substituted benzamidines, none of the *N*-mono- and *N,N*-disubstituted representatives possessing hydrogen atoms on a carbon adjacent to the nitrogen (α-H-atoms) was *N*-oxygenated /28/. However, when the *N-tert.*-butylbenzamidine 5, which does not possess α-H-atoms was examined, the *N*-oxygenation to give the corresponding amidoxime 6 was observed (Equation (3)) /29/.

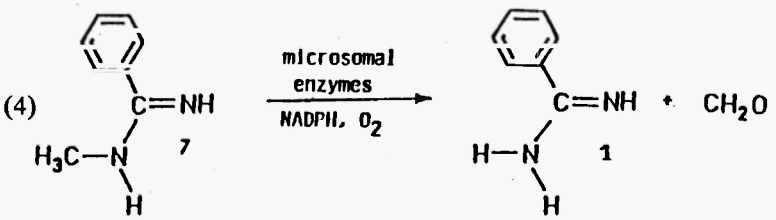
(3) 

This reaction (Equation (3)) demonstrates that the active site of the cytochrome P-450 form involved can accept substrates with bulky substituents and suggests that steric aspects may be of minor significance. They, however, cannot be neglected completely since the

rate of reaction of the *N-tert.*-butylbenzamidine is low and moreover the even more bulky *tert*-octyl analogue is not *N*-oxygenated /29/.

3.3 N-Demethylation of N-Methylbenzamidine

The postulate – *N*-dealkyation of benzamidine with α-H-atoms /11/ was confirmed for *N*-methylbenzamidine 7 which reacted to form benzamidine 1 and formaldehyde (Equation (4)) /24/. This biotransformation of a strongly basic amidine was demonstrated for the first time, the reaction previously being known only for the slightly basic chlorophenamidine /30/.



Characterisation of the *N*-demethylation reaction of *N*-methylbenzamidine gave results similar to those obtained for the *N*-oxygenation of benzamidine (see Table 1). The participation of the cytochrome P-450 enzyme system was again considered as probable on the basis of experiments with inhibitors and, in particular, carbon monoxide (see Table 2) /24/.

3.4 Mechanisms and Conclusions for N-Oxygenations of Bentamidines

One of the objectives of these investigations was to make a contribution to the problem of the predictability of biotransformation processes. For *N*-oxidative processes, it was postulated that an *N*-oxygenation can only take place when an *N*-dealkylation is not possible because of an absence of α-hydrogen atoms /11/.

This is in agreement with a radical mechanism for the reaction with cytochrome P-450 (see Figures 4 and 5). In the case of unsubstituted benzamidines (Figure 4), an electron can be taken up by the active iron-oxygen complex in the last step of the reaction cycle with

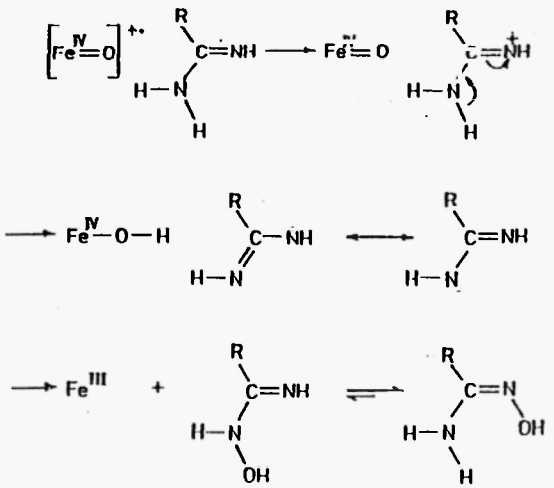


Fig. 4: Proposed mechanism for the N-oxygenation of benzamidines without -hydrogens /11/.

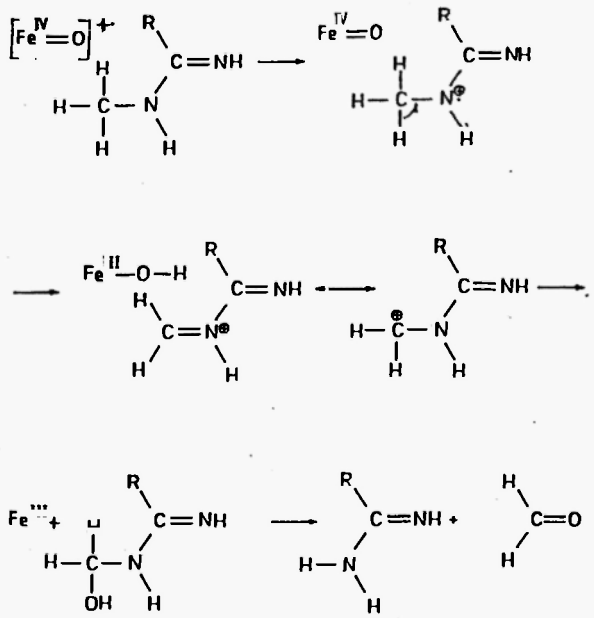


Fig. 5: Proposed mechanism for the C-oxygenation of N-alkylsubstituted benzamidines with -hydrogens.

cytochrome P-450 /31/. A radical cation is thus formed, which can be stabilised either by the cleavage of a proton, as formulated in Figure 4 or by the removal of a hydrogen radical. The former process produces a radical which is stabilised by mesomerism, and this can be hydroxylated to give an hydroxyamidine which finally tautomerises to furnish the amidoxime /11/. The same mechanism could also be in operation for *N*-phenylbenzamidine and *N-tert.*-butylbenzamidine.

In the case of the *N*-alkylbenzamidines with α-hydrogens such as *N*-methylbenzamidine (see Figure 5), a carbenium-iminium ion could be formed by the cleavage of a hydrogen radical after removal of an electron. In this way, an hydroxyl group can be transferred to the carbon atom to form a carbinolamidine. The latter decomposes immediately to give the *N*-dealkylated benzamidine and formaldehyde. Such mechanisms have also been discussed for the *N*-dealkylation of amines /32/. The net result is therefore that *N*-alkylbenzamidines are *N*-dealkylated rather than *N*-oxygenated.

Our studies on structure-activity relationships in the *N*-oxidative metabolism of amidines (see above) provide experimental confirmation of the mechanisms explained above and show that *N*-oxygenation instead of *N*-dealkylation of nitrogen-containing functional groups by the cytochrome P-450 enzyme system can be expected when no α-hydrogen atoms are available. The results can be summarised as follows:

a) *N*-Oxygenation (Reaction (1)) and *N*-dealkylation (Reaction (4)) exhibit comparable characteristics. The characteristics of oxygen- and NADPH-dependency required for microsomal monooxygenase can be seen for both reactions. The inhibitors, especially carbon monoxide, confirm the participation of cytochrome P-450 in each case. Induction experiments indicate that the isoenzymes involved are not induced by phenobarbitol, 3-methylcholanthene ethanol or benzamidines /23, 24/.

   Catalase or superoxide dismutase have no effect on the reactions; this means an H_2O_2-supported enzymatic *N*-oxygenation or *N*-dealkylation ('peroxidative function of liver microsomal cytochrome P-450'), as has been described for oxidative *N*-dealkylation reactions /33/, or a superoxide-mediated oxidation, as has been reported for *N*-hydroxyphentermine /34/, can be discounted. Furthermore, oxy species were not capable of transforming benzamidines /23/.

The question arises of whether the same cytochrome P-450 isoenzyme is responsible both for the *N*-oxygenation (Reaction (1)) and for the *N*-demethylation (Reaction (4)). In particular, the similar behaviours towards inducers and the inhibitor carbon monoxide at low inhibitor concentrations are in favour of the former. However, at higher CO/O_2 values, a larger sensitivity is determined for the *N*-demethylation than for the *N*-oxygenation (see Table 2 for $CO/O_2 = 5$ and $CO/O_2 = 8$). Of the other inhibitors, SKF 525 A and KCN behave similarly whereas the *N*-demethylation but not the *N*-oxygenation is significantly inhibited by metyrapone($P < 0.05$).

Both reactions follow Michealis-Menten kinetics /23 24/. However, the apparent K_m and V_{max} values determined from the Lineweaver-Burk graphs /23,24/ deviate from one an other (1.95 ± 0.30mM and 0.54 ± 0.17nmol/min/mg of protein for *N*-oxygenation compared with 23.8 ± 4.2mM and 1.98 ± 0.23 nmol/min/mg of protein for *N*-demethylation, means of determinations from three rabbits ± S.D.). Thus, further investigations are required to determine conclusively whether the same isoenzymes of cytochrome P-450 are responsible both for *N*-oxygenation (Reaction (1)) and for *N*-demethylation (Reaction (4)).

This question is, however, not of importance for the concept presented here. It is now clear that the various cytochromes P-450 so far examined in detail are discrete gene products; that is, they differ in their amino acid sequences /35/. The catalytic course of oxygen activation is, however, identical for all microsomal cytochrome P-450 isoenzymes /31/, hence the mechanism of *N*-oxygenation and the mechanism of *N*-dealkylation with widely different isoenzymes also should not differ. If such a catalytic mechanism is in operation, the product formation (*N*-oxygenated or *N*-dealkylated product) should depend principally on the presence or absence of α-hydrogen atoms. This is not only valid for amidines but also for other functional groups (see below). It must be considered here, however, that the concept *N*-oxygenation or *N*-dealkylation depending on the presence or absence of α-hydrogen atoms cannot make any predictions as to whether or not the respective compound can be accomodated at the substrate binding site of the widely differing cytochrome P-450 isoenzymes.

b) For *N*-substituted benzamidines where α-hydrogen atoms are absent, as in the case of *N*-phenylbenzamidine /28/ and *N-tert.* butylbenzamidine /29/, an *N*-oxygenation occurs also in accordance with the postulated mechanism (Reactions (2) and (3)) These *N*-oxygenations cannot be attributed to the lipophilicities of these compounds since other benzamidines with lipophilic groups such as *N,N*-dimethylbenzamidine, *N,N*-pentamethylenebenzamidine, and *N*-methyl-*N*-phenylbenzamidine were not *N*-oxygenated /28/.

c) These considerations lead to the assumption that the presence of electron donors on the ring renders the removal of an electron from the amidine system more easy, whereas the presence of electron acceptors makes it more difficult. This assumption was confirmed for the examples of some *para*-substituted benzamidines where the logarithms of the maximum reaction rates showed a linear correlation with the Hammett constants (see Figure 3) /27/. The experimentally determined ρ value (see Figure 3) serves to support the proposed mechanism for the *N*-oxygenation of amidines, since reactions with negative /36/ or low /37/ values of ρ often proceed by way of radical intermediates. It was considered unlikely that the differing reaction rates were the result only of changed basicities /27/.

IV. N-OXYGENATIONS OF OTHER FUNCTIONAL GROUPS

The concepts presented here for the possibility of *N*-oxygenation by cytochrome P-450 are based on mechanisms and structural features /11/. This is in agreement with the conclusions of other authors who also assumed an *N*-oxygenation by this enzyme system in the absence of α-H-atoms /9,10/. Their considerations /9,10/ were based on reactions of primary amines, amides, and pyridines reported in the literature. The same concept has been deduced independently from our investigations on amidines /11/ and has been confirmed in recent years by the series of investigations summarised in this review.

A further functional group lacking α-H-atoms for which an *N*-oxygenation has been described is represented by the ketimines which are converted to oximes /15/. In this case as well, all evidence is in favour of the participation of cytochrome P-450 /16/. From purely

formal considerations, the *N*-oxygenation of amidines (Reaction (1)) might be compared to that of the imines /15/. However, it is immediately apparent from the mechanism (see Figure 4) that both nitrogens of the amidine are involved and not just the doubly bonded nitrogen atom. Induction experiments show that, in contrast to the *N*-hydroxylation of imines /16/, the *N*-oxygenations of amidines are not significantly induced by phenobarbital /23/. Thus, it would seem that other isoenzymes are also involved. These differences, however, are not surprising when the chemical differences between imines and amidines are taken into account (see Chapter 2.1) and it is recognised that the double bond in the amidines is delocalised.

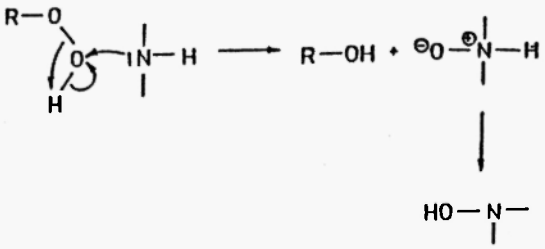
Although it is practically certain that the majority of functional groups susceptible to *N*-oxygenation by cytochrome P-450 are those without α-H-atoms, there are exceptions in the case of nitrogen radicals which have increased stabilities (e.g. azoprocarbazine /9,38/) and of bicyclic systems (e.g. norcocaine /39/ and quinidine /40/). However, for the bicyclic systems Bredt's rule applies /40/, and – in agreement with the proposed mechanism (see Figure 5) – the elimination of α-hydrogen radicals to form iminium ions may not be favoured. Thus, in spite of α-hydrogen atoms, the oxygen is transferred to the nitrogen.

Exceptions which confirm the rule may be supplied by the amines tiaramide /41/ and dibenzylamine /42/, for which, in spite of the presence of α-hydrogen atoms, evidence is available for *N*-oxygenation not only by cytochrome P-450 but also by the flavine-containing monooxygenase /41, 42/.

Within the group of amphetamines, *N*-oxygenation of phentermine (without α-H-atoms) by cytochrome P-450 has been demonstrated /34/ In the case of methamphetamine (with α-H-atoms) however, involvement of not only cytochrome P-450 but also flavine-containing monooxygenase has been considered possible /43/. For such amines and also for hydroxylamines both *N*-hydroxylation proceeding in the normal way and one in which the substrate uncouples the cytochrome P-450, thus generating superoxide and hydrogen peroxide which then transform the substrate, are feasible /34/. Thus, phentermine is *N*-hydroxylated by cytochrome P-450 in the normal way while the further transformation of the thus formed hydroxylamine proceeds by way of superoxide generated from cytochrome P-450 via uncoupling and, possibly, by a peroxidatic pathway involving cytochrome P-450 and hydrogen peroxide generated by uncoupling /34/. Since other mechansims are in operation, the latter transformations cannot be covered by the present concept.

A large number of nitrogen functional groups are transformed by the flavine-containing monooxygenase /44/. The *N*-oxygenation consists of a direct, two-electron oxidation of the nitrogen atom by means of the transfer of an oxygen atom from the peroxyflavine bonded to the enzyme /45, 46/. The details of the catalytic mechanism of this monooxygenase are well known. The transfer of oxygen from peroxyflavine is comparable with the *N*-oxygenation of amines by peroxides /10/. The transfer of oxygen to a nitrogen atom results in the formation of an *N*-oxide which, when hydrogen atoms are still bonded to the nitrogen, undergoes tautomerism to give the hydroxy compound (Equation (5)) or, as in the case of hydrazines, undergoes cleavage of water /44/.

(5)



According to this mechanism which lacks radical intermediates, alkyl groups with α-H-atoms should not have a significant influence on the *N*-hydroxylation. In agreement with this, flavine-containing monooxygenase can *N*-oxygenate monomethyl- and dimethylamines /10/. On this basis, it would appear that this enzyme cannot be involved in the *N*-hydroxylation of benzamidines. Experiments with the purified enzyme have provided confirmation that this is not so /12/. It may be that the nucleophilicity of the amidine in aqueous solution is too low for it to attack the peroxyflavine. Our experiments with peracids have shown that amidines are not transformed under such conditions.

Further aspects of substrate specificity for the flavine-containing monooxygenases will not be discussed in detail here since excellent reviews have been published, the most recent being /44/. According to /44/, the hog liver enzyme, which has been most thoroughly investigated, is able to transform tertiary amines to stable *N*-oxides and secondary amines to hydroxylamines which may undergo further reactions. Hydrazines are also substrates. In addition to amidines /12/ and guanidines /47/, amides, carbamines, imines, nitrones, oximes, isocyanates, nitriles, and nitrogen-containing aromatic heterocycles are also not attacked by the peroxyflavine /44/.

V. CONCLUSIONS AND PERSPECTIVES

If metabolic *N*-oxygenation of a specific functional group within a molecule is to be predicted, the first question to be answered is the relative likelihood of reaction with cytochrome P-450 or the flavine-containing monooxygenases. In the light of what has been said, susceptibility to *N*-oxygenation by peracids /44/ may be a useful criterion. However, if the answer to this question is yes, it still has to be clarified whether the compound is accessible to the enzyme /44/. This second question can only be answered by experiment since very little is yet known in this respect for the flavine-containing monooxygenase Moreover, further variants (especially rabbit lung enzyme) of the enzyme have been investigated and found to possess the same catalytic mechanism but somewhat different specificities /44/.

For *N*-oxygenation by cytochrome P-450, reaction can be realised especially when no α-H-atoms are present. If α-H-atoms are present, the formation of *N*-dealkylated products predominates. However, because of the possibility of participation of widely differing isoenzymes, only experiments can reveal whether or not the steric prerequisites for a suitable bonding of the substrate to the respective isoenzyme are present.

The situation is rendered more complicated by the fact that enzymic or purely chemical reactions of the initially-formed *N*-oxygenated compounds have to be taken into account. In particular, the possibility of metabolic retroreductions of *N*-oxygenated compounds must be considered when working with non-purified enzymes and in the case of *in vivo* studies /44/. Thus, in the course of investigations in my laboratory, the reduction of benzamidoxime to benzamidine by microsomal fractions has been recently demonstrated /48/. Although the *N*-oxygenations discussed here clearly dominate with the respective rabbit enzyme sources, the picture is more complicated when rat enzyme sources are employed. Metabolic cycles of this type will be the subject of future investigations.

In addition, we will also address the question concerning which of the two nitrogen atoms within the amidine functional group receives the transferred oxygen atom. In the case of unsubstituted amidines this question is irrelevant since identical products are formed but with *N*-substituted amidines, however, two *N*-oxygenated products can be

formed and these may exhibit different pharmacological and toxicological properties.

The fact that genotoxicity may be dependent on the site of the *N*-oxygenation in molecules containing several nitrogen atoms has been demonstrated by Gorrod for the example of amino-substituted nitrogen-containing aromatic systems /49/. The examples cited /49/ possess an exocyclic amino group attached to a carbon atom that is bonded to at least one further nitrogen atom in the aromatic ring. Although such compounds cannot be classified as typically basic amidines (one of the nitrogen atoms is a component of an aromatic ring system), the possible sites of *N*-oxygenation within these related systems are at present being investigated.

Of the criteria which have to date been held responsible for determining product formation /49/, better access of one or the other nitrogen atom of an amidine to the oxygen atom bonded to the enzyme seems to be the decisive factor. Depending on the participating isoenzyme of cytochrome P-450, different bonding prerequisites could exist. The tautomerism under discussion /8,49/ seems to us to be unimportant for the variations in product formation.

N-Hydroxylation can be of pharmacological and toxicological relevance in the case of strongly basic amidines. Thus for the pentamidine, it has also been shown that the corresponding amidoxime also possesses a high trypanocidic activity /50/.

Similarly, benzamidoxime induces DNA single-strand breaks (in rat hepatocytes) and DNA amplification in SV40-transformed hamster cells /51/. For these reasons, the studies on the biotransformations of amidines and their metabolites will be continued.

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