

SOME STUDIES ON DIRECTED AROMATIC HYDROXYLATIONS

A thesis submitted by

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ai miei genitori, per la pazienza e l'affetto durante
il lungo corso della mia educazione

ABSTRACT

Review :

The enzymes fixing oxygen to aromatic substrates are briefly defined. The mechanistic meaning of the NIH shift is considered. Some chemical models, showing NIH shift or believed to proceed via atomic oxygen, are examined. The photochemical behaviour of aromatic N-oxides is summarised, particular stress being given to the oxidation reaction of aromatic substrates on irradiation in the presence of aromatic N-oxides.

Discussion and experimental :

The irradiation of pyridine N-oxide in benzene has been repeated, in order to check the yield of phenol formed in the reaction. A quantitative analysis by u.v. spectroscopy and by formation of 2,4,6-tribromophenol has been carried out. The literature yields appear to be higher (15% versus 1%). The irradiation of pyridine N-oxide boron trifluoride in benzene gave an enhanced yield of phenol (7%). 2-Phenethylpyridine N-oxide and 2-benzylpyridine N-oxide were irradiated, in order to favour an intramolecular hydroxylation process, particularly in the ortho position. The rearrangement products obtained via the formation of an oxaziridine type intermediate and the production of tars were the prevalent processes, although small amounts of the respective o-phenols could be detected.

The irradiation of the boron trifluoride complexes of 2-phenethylpyridine N-oxide and of 2-benzylpyridine N-oxide was examined. The rearrangement products obtained via the oxaziridine appeared to have been virtually suppressed and the formation of tars was diminished. The directed hydroxylation (ortho) yields improved remarkably and benzylic oxidation was also observed. The boron trifluoride complex of 2-phenoxy pyridine N-oxide was similarly irradiated and meta and para hydroxylated products were detected, although the ortho hydroxylation was largely prevalent. Solutions of different concentrations of 2-phenoxy pyridine N-oxide boron trifluoride were irradiated and the o-, m-, p- product relative ratios tentatively calculated by h.p.l.c.-u.v. technique ; the experimental error associated with this technique is too large and therefore this experiment was not conclusive to decide whether an inter or intramolecular oxygen transfer mechanism operates. 2-([2',4',6'-²H₃]Phenoxy) pyridine N-oxide was synthesised. Its boron trifluoride adduct was irradiated and the o- and p-phenols obtained showed a considerable deuterium retention, while the m-phenol showed a lower deuterium retention. Therefore it is concluded that such hydroxylation occurs with the NIH shift and that arene oxides are formed as intermediates.

A method for synthesising [¹⁸O]-labelled aromatic N-oxides from ¹⁸O₂ gas is described ; such compounds have never, as far as known, been previously synthesised.

The boron trifluoride adducts of 2-phenoxy pyridine-

$[^{18}\text{O}]$ -N-oxide and of 2-([2',4',6'- $^2\text{H}_3$]phenoxy) pyridine N-oxide were irradiated. No crossed product (with one ^{18}O and three ^2H) could be detected for the o-phenol. The o-hydroxylation therefore appears to proceed via an intramolecular oxygen transfer mechanism and via formation of an arene oxide.

‘ ‘ O frati, ‘ dissi, ‘ che per cento milia
perigli siete giunti all’occidente,
a questa tanto picciola vigilia

de’ nostri sensi ch’e’ del rimanente,
non vogliate negar l’esperienza,
di retro al sol, del mondo senza gente.

Considerate la vostra semenza:
fatti non foste a viver come bruti,
ma per seguir virtute e canoscenza.’

Li miei compagni fec’io sì aguti,
con questa orazion picciola, al cammino,
che a pena poscia li avrei ritenuti;

e volta nostra poppa nel mattino,
dei remi facemmo ali al folle volo,
sempre acquistando dal lato mancino.

Dante Alighieri

“La Divina Commedia”, Inferno, Canto XXVI, 112-126

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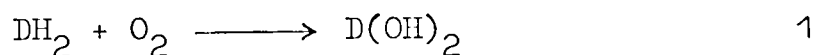
R E V I E W

1. OXYGENASES AND MONOOXIDASES ⁽¹⁾

The hydroxylation of aromatic compounds is catalysed in vivo by enzymes named oxygenases. These enzymes intervene at essential points in the metabolism of certain amino acids, porphyrins, lipids and steroids. Oxygenases also participate in the animals in the catabolism and detoxification of drugs and other substances. Many detoxification processes take place in the liver, and a key step is the hydroxylation of certain noxious aromatic compounds.

The insertion of oxygen atoms, derived from molecular oxygen ⁽²⁾, is catalysed by these enzymes. Two subclasses of oxygenases can be distinguished :

a) The dioxygenases, also called oxygen transferases. They catalyse the insertion of both atoms of molecular oxygen (reaction 1) :



b) The monooxygenases, also called hydroxylases, or mixed function oxidases, which introduce only one oxygen atom into the substrate (reaction 2) :



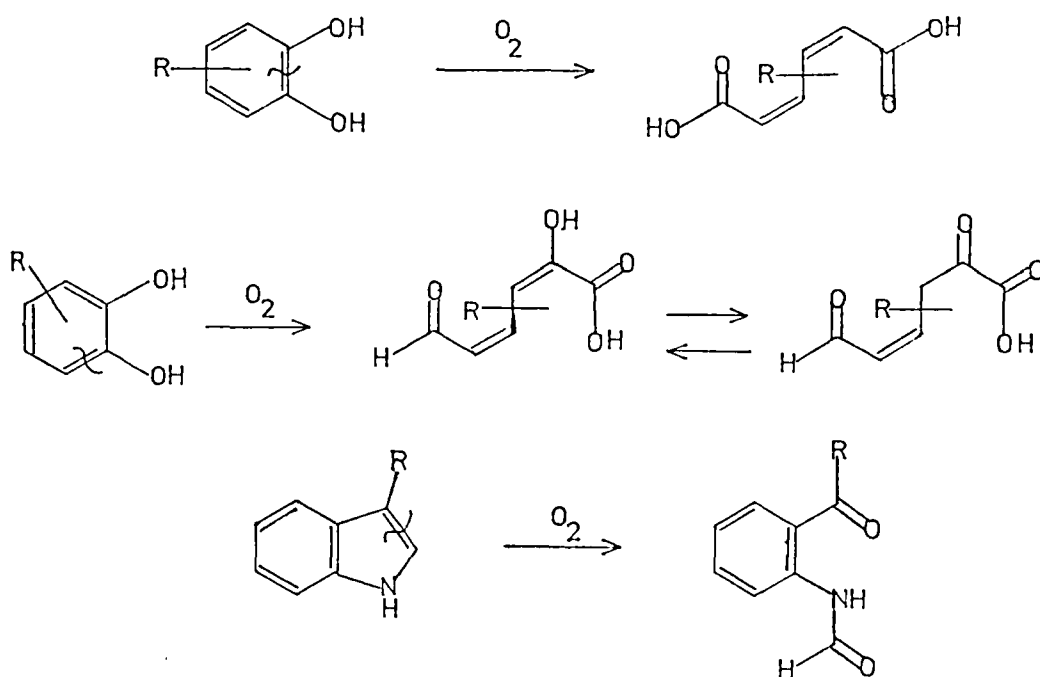
Dioxygenases

These oxygenases are responsible for the introduction of two oxygen atoms into the substrate : a dihydroxy derivative is formed as the first product. The dioxygenases generally

catalyse three main types of reactions:

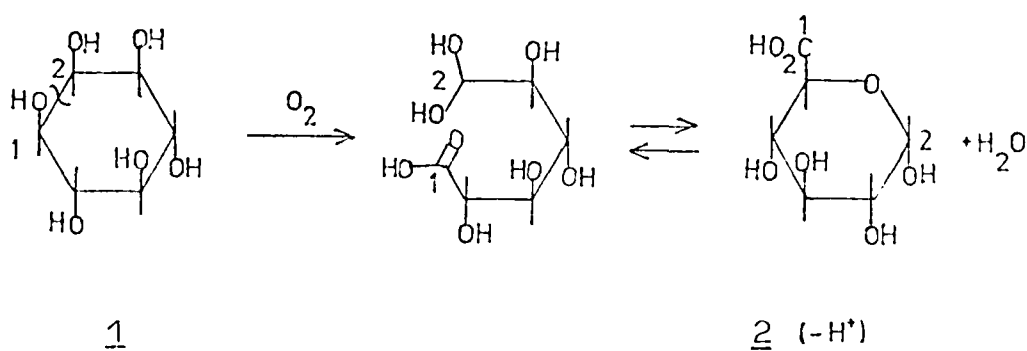
1) Cleavage of aromatic rings: aromatic double bonds, located between two phenolic groups or adjacent to single phenolic groups, or in an indole ring, are cleaved (Fig. 1).

Fig. 1



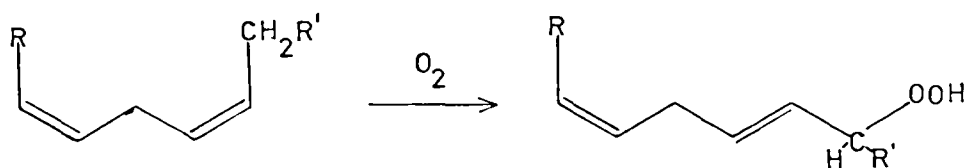
2) Oxygenation of aliphatic structures: myo-inositol 1 is converted to D-glucuronate 2 (Fig. 2).

Fig. 2



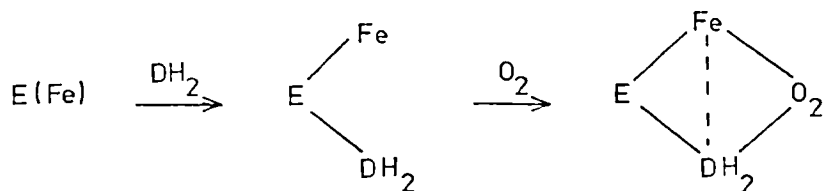
3) Hydroperoxidation reactions: these reactions are catalysed by lipoxygenases, and are prevalent in the plant kingdom. Lipids containing a cis,cis -1,4-pentadiene system are converted to the corresponding hydroperoxides having a cis,trans -configuration (Fig. 3).

Fig. 3



The nature of the prosthetic group activating the oxygen is unknown for this last case: in some of the other enzymes there is a good evidence for the participation of Fe^{2+} . Several dioxygenases have now been crystallised or greatly purified: iron appears to form part of the prosthetic group in all instances. Examples are metapyrocatechase, a protein with M.W. 140 000 containing three active sites, each incorporating one Fe^{2+} ion, and the protocatechuate-3,4-dioxygenase, with M.W. 700 000 and eight Fe^{3+} ions at an equal number of active sites. Iron chelating agents inhibit the reactions catalysed by these enzymes. A schematic but undetailed mechanism applicable to all the dioxygenases so far examined is summarised in Fig. 4.

Fig. 4



The reaction is presumed to take place within a ternary complex of iron enzyme, substrate and oxygen. The latter is supposed to be linked to both the prosthetic metal and the substrate. No oxygenated complexes are supposed to be formed in the absence of the latter, but recently some exceptions have been found.

Hydroxylases

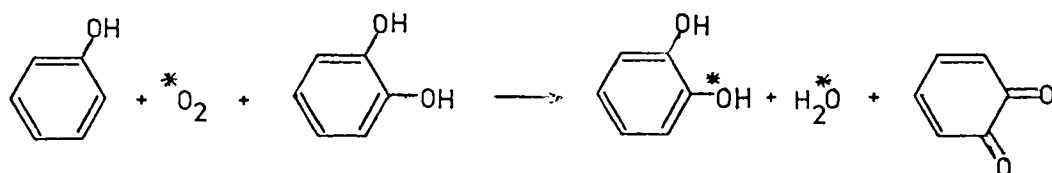
The stoichiometry of the reaction catalysed by this class of enzymes is described in reaction 2 : a single atom of oxygen is inserted. The appearance of labelled oxygen in water as well as in the product, and the absolute requirement for an oxidisable cosubstrate (XH_2) distinguish this reaction from the ones catalysed by dioxygenases. Depending on whether the obligatory cosubstrate is itself a product of the reaction, monooxygenases have been classified as internal or external.

(i) Internal monooxygenases

Monophenols are converted to diphenols, and the latter are dehydrogenated to o-quinones: these reactions occur in

plants, and are catalysed by some copper-containing enzymes. The two classes of reaction are usually referred to as the cresolase (monophenol oxidase) and catecholase (diphenol oxidase) types of the so-called phenolase complex (Fig. 5).

Fig. 5



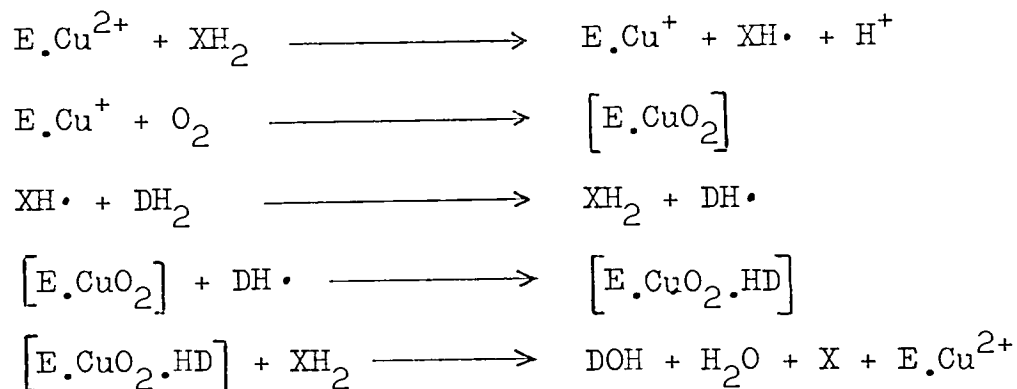
The diphenol cosubstrate is itself a product of the reaction. The overall reaction is the conversion of a monophenol to an o-quinone, after being first hydroxylated and then dehydrogenated. The oxygen molecule is split during the reaction: one atom is reduced to water, and the other is used to hydroxylate the aromatic ring. The diphenol cosubstrate is not absolutely required in this case: other reducing agents, such as ascorbate or hydroquinone, can participate in its stead - the oxygenation is in that case external rather than internal.

(ii) External monooxygenases

One example is given by mammalian dopamine hydroxylase, which is a copper protein that requires ascorbate as the obligatory cosubstrate. The following mechanism has been deduced ⁽³⁾ (Fig. 6).

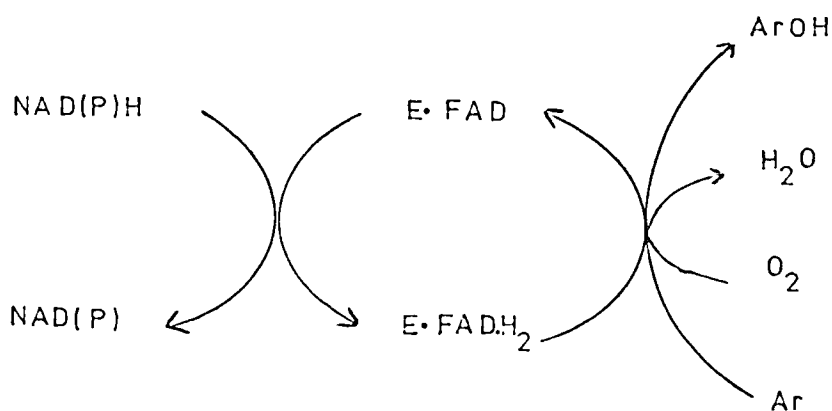
Fig. 6

(XH_2 = ascorbate ; DH_2 = dopamine)



Nicotinamide nucleotide coenzymes are used as ultimate hydrogen donor or cosubstrate in most other hydroxylases. The number and kind of steps intervening differ greatly in complexity. Certain bacterial hydroxylases show the simplest pattern: they are single proteins, with a flavin unit as the prosthetic group; an example is the *p*-hydroxybenzoate hydroxylase of Pseudomonas (Fig. 7).

Fig. 7



NAD(P) = nicotinamide adenine dinucleotide phosphate
(oxidised form)

NAD(P)H = nicotinamide adenine dinucleotide phosphate
(reduced form)

FAD = flavin adenine dinucleotide (oxidised form)

FAD.H₂ = flavin adenine dinucleotide (reduced form)

The reduced flavin acts as the proximal hydrogen donor for the hydroxylation of the substrate. The reduction of this entity and the hydroxylation of the substrate are catalysed by the same enzyme. In some instances binding of substrates precedes the reduction of E.FAD : a ternary complex between both substrates and the enzyme is an intermediate in the reaction.

A more complex case is when the proximal hydrogen donor is reduced by one protein, while it serves as a cosubstrate for another (the actual hydroxylase). The phenylalanine hydroxylase of mammalian liver is such an example. A pteridine here acts as a link between a reductase on the one hand, and the hydroxylase on the other (Fig. 8).

A very important feature in this reaction is the shift of the p-hydrogen to the m-position of the product (L-tyrosine 3) : such a shift is called the NIH shift because it was discovered at the National Institutes of Health ^(4,5). As will be described later, this discovery has an important mechanistic consequence, namely the formation of arene-oxides 4 as intermediates of the in vivo hydroxylation of aromatic compounds (Fig. 9).

Fig. 8

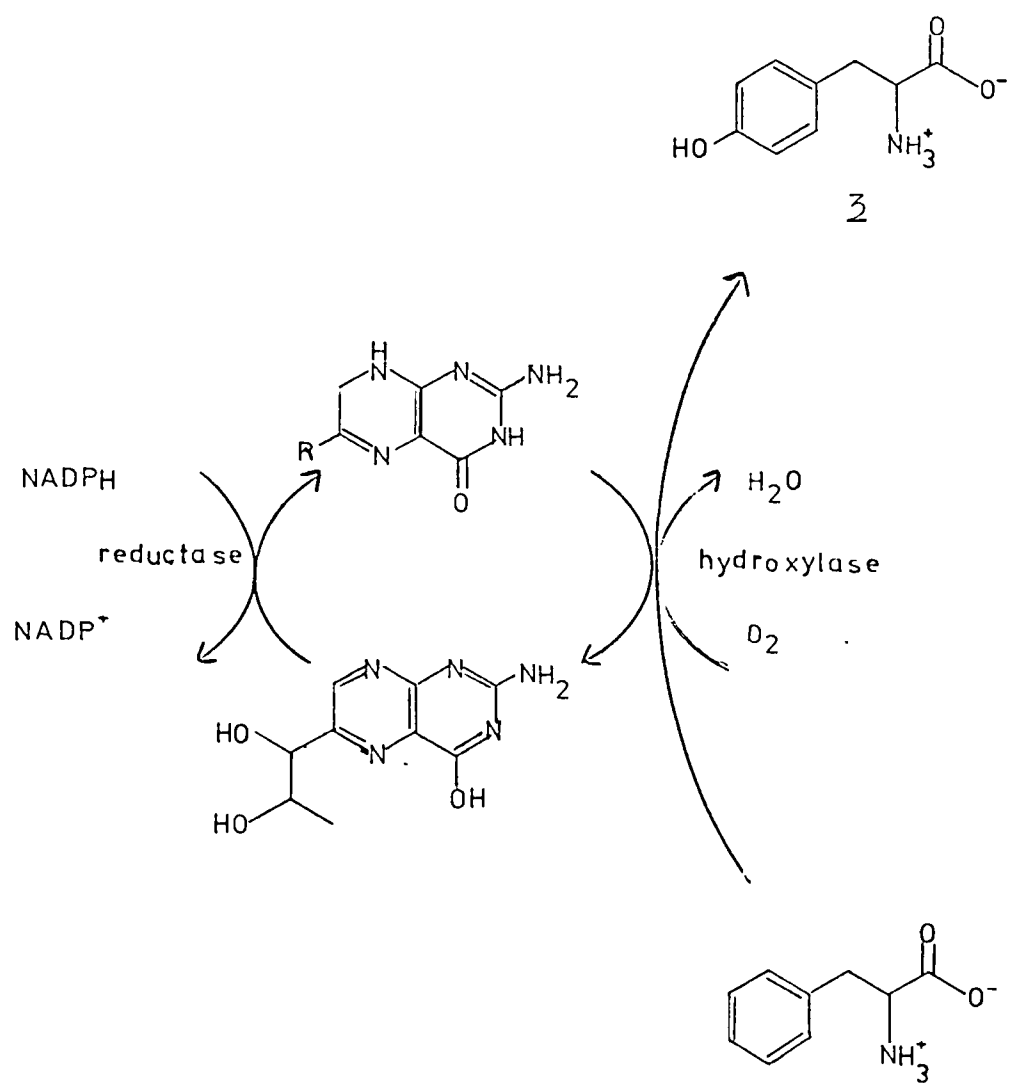
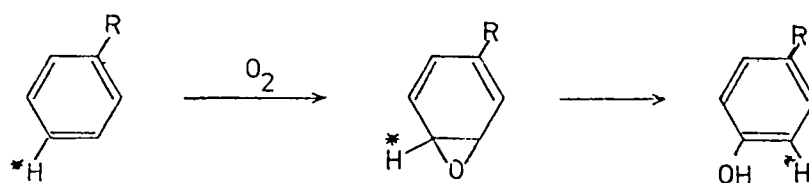


Fig. 9

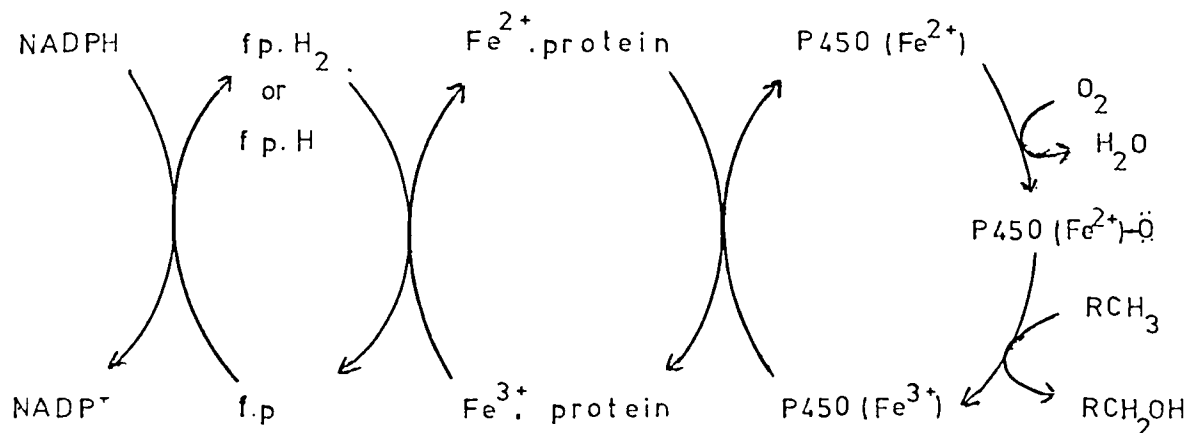
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The more complex stage is reached with a group of hydroxylases localised largely in the microsomal fractions of vertebrate organs, such as the liver, the adrenal glands and the female sex glands. As already mentioned the liver enzymes are principally concerned with the metabolism and detoxification of a large number of aromatic compounds, and with key steps in lipid metabolism. Examples of the former type are the conversion of naphthalene to 1,2-dihydro-1,2-dihydroxy naphthalene, of L-kinurenine to L-3-hydroxy-kinurenine, of tyrosine to 3,4-dihydroxyphenylalanine (DOPA) and of tryptamine to 6-hydroxytryptamine.

A haemoprotein with an absorption maximum at 420 nm (that of its CO derivative is at 450 nm), called B 420 (or P 450) occurs widely in microsomal and mitochondrial fractions of animal tissues. It has been shown to function as the terminal oxidase in the conversions catalysed by a variety of NAD(P)H mixed function oxidase systems, such as those concerned with drug metabolism and bacterial hydroxylations linked to NADH. The immediate electron donor in all cases is provided by a reduced non-haeme iron (NHI) protein, called adrenodoxin, when of animal origin, and rubredoxin when of bacterial origin. The ultimate electron donor NAD(P)H in turn reduces the NHI protein, with the intervention of a flavoprotein reductase (Fig. 10).

In this formulation a reactive oxygen bound to ferrous ion, such as the ferryl ion complex $(\text{Fe}^{2+})-\ddot{\text{O}}$ or $(\text{Fe}^{4+})-\text{OH}$ is postulated as the reactive species in the oxygenation reaction proper.

Fig. 10

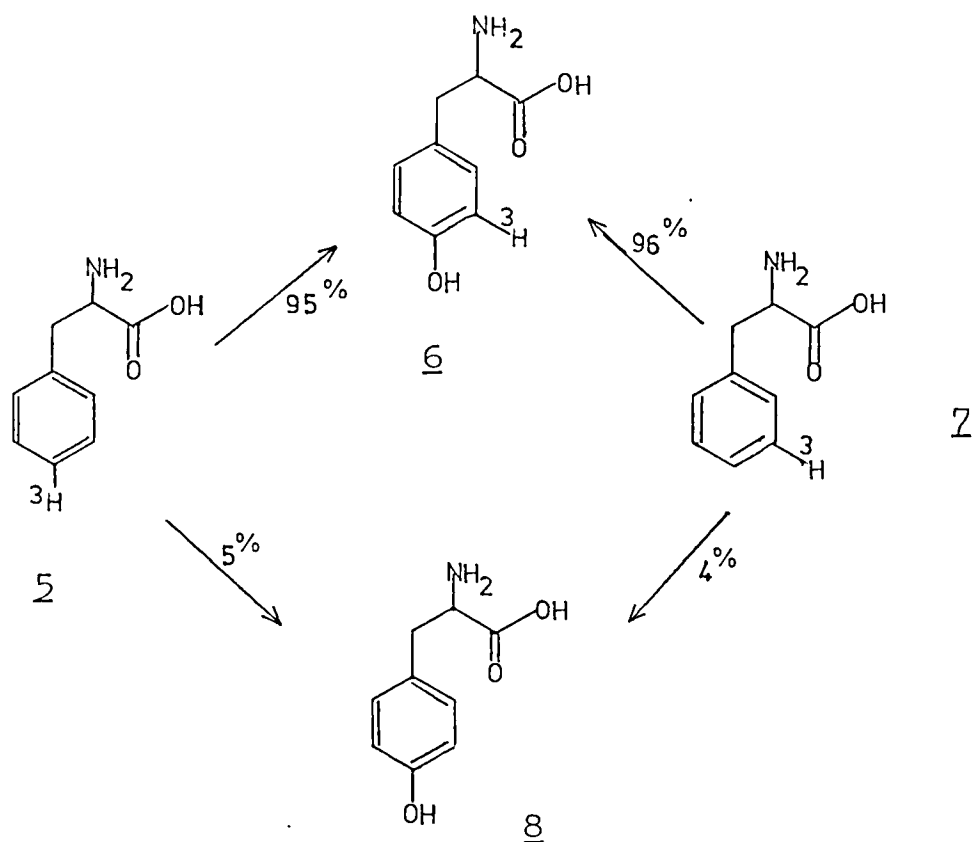


f.p. = flavoprotein

2. THE NIH SHIFT IN THE IN VIVO HYDROXYLATION. THE ARENE OXIDES. THE AROMATISATION MECHANISM.

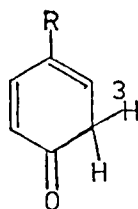
The in vivo hydroxylation of aromatic compounds has been extensively studied. Various tritiated substrates have been used and usually a stoichiometric amount of tritium is liberated from the substrate; proline (6), tyrosine (7) and dopamine (8) hydroxylases can be rapidly assayed in such a way. When tyrosine was formed from $[4-^3\text{H}]$ -phenylalanine 5, 95% of the tritium was retained (4) (Fig. 11); $[4-^3\text{H}]$ -phenylalanine 5 gave $[3-^3\text{H}]$ -tyrosine 6 with 95% of tritium

Fig. 11



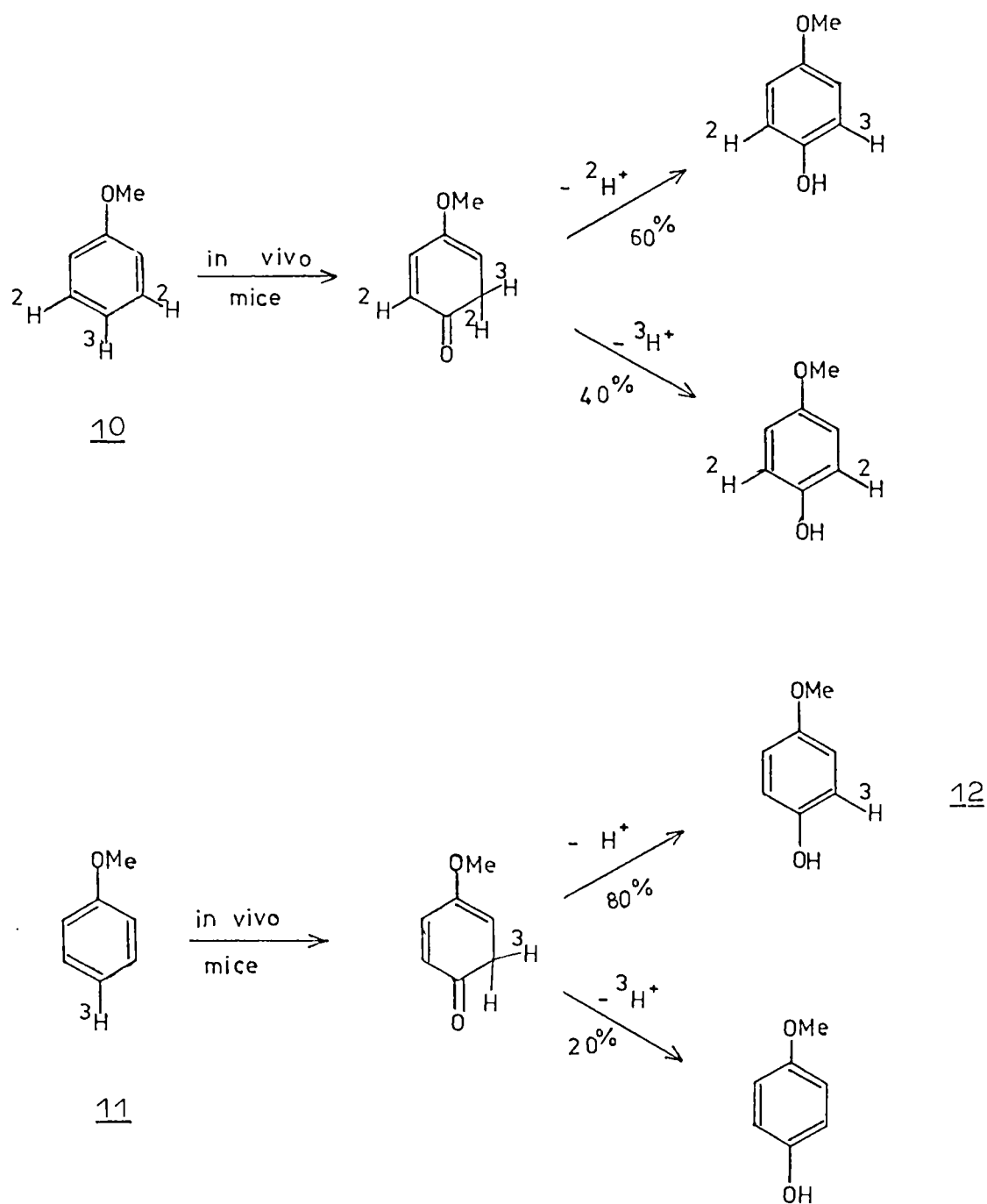
retention ; $[3-^3\text{H}]$ -phenylalanine **2** is enzymatically hydroxylated to $[3-^3\text{H}]$ -tyrosine **6** and to tyrosine **8** with the same ratio. This indicates a common intermediate **9** (Fig. 12), which aromatises losing a proton rather than tritium, because of the kinetic isotope effects.

Fig. 12

**9**

Kinetic isotopic effect studies have been carried on $[3,5-^2\text{H}_2;4-^3\text{H}]$ -anisole 10 and $[4-^3\text{H}]$ -anisole 11 ⁽⁹⁾ (Fig. 13).

Fig. 13



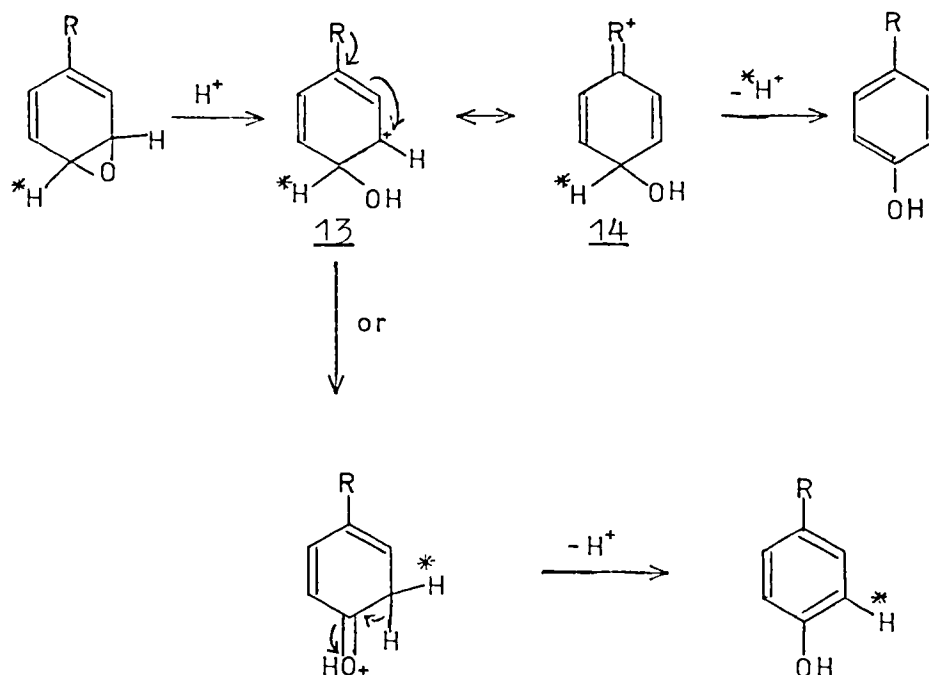
The type and extent of kinetic isotope effect operating is well illustrated in the example outlined in Fig. 13 . When tritium is flanked by two deuteriums, such as in 10 , a tritium retention of 60% is observed ; a tritium retention of 80% is observed in compound 12 .

Most of the in vivo hydroxylation studies have been carried on with liver microsomes. The hydroxylating system is probably a mixture of enzymes including the previously mentioned cytochrome P 450 : such a system is not as specific as the soluble phenylalanine and tryptophan hydroxylases. It usually catalyses most detoxification processes in the liver, by introduction of the hydroxyl group. Further reactions increase the water solubility of the catabolites so that the final products can be excreted in the urine.

Another important feature of such hydroxylation is the electrophilic character shown by the attacking hydroxylating species. Various experimental results have indicated an attack by active oxygen on the π electron system of the aromatic hydrocarbon. The ratio of o-,m- and p-cresols obtained by the in vivo microsomal oxidation of toluene is 59:13:28 and benzyl alcohol is also obtained ⁽¹⁰⁾ .

The substituent R strongly influences the extent of migration and retention of the hydrogen, according to its capability of stabilising the intermediate cyclohexadiene 13 by conjugation effects as in intermediate 14 (Fig. 14). The cross-conjugated cyclohexadienol 14 can rearomatise only if the hydrogen in the p-position is lost as a proton. The low retentions of deuterium (30 or 40%) and tritium (40 or 60%)

Fig. 14

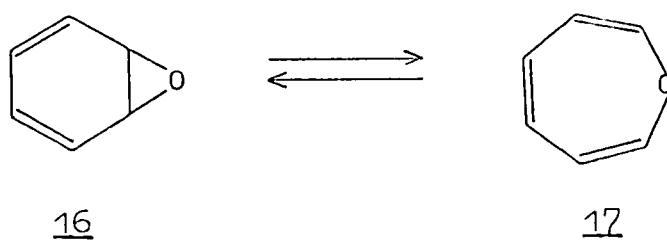


shown in vivo or in vitro by anilides is attributed to such a reaction ⁽¹¹⁾.

Arene oxides were postulated as intermediates in the metabolism of aromatic compounds as early as 1950 ⁽¹²⁾. The first arene oxide metabolite to be isolated, some twenty years later on, was the tricyclic naphthalene-1,2-oxide 15 ⁽¹³⁾. A convenient definition of arene oxides has been given by Jerina, Yagi and Daly in a recent review ⁽¹⁴⁾: arene oxides are a class of aromatic hydrocarbons in which the continuous π -overlap is interrupted by an epoxide ring between two adjacent carbon atoms.

The parent member of such a series is benzene oxide 16, which can exist in valence tautomerism with oxepin 17 (Fig. 15).

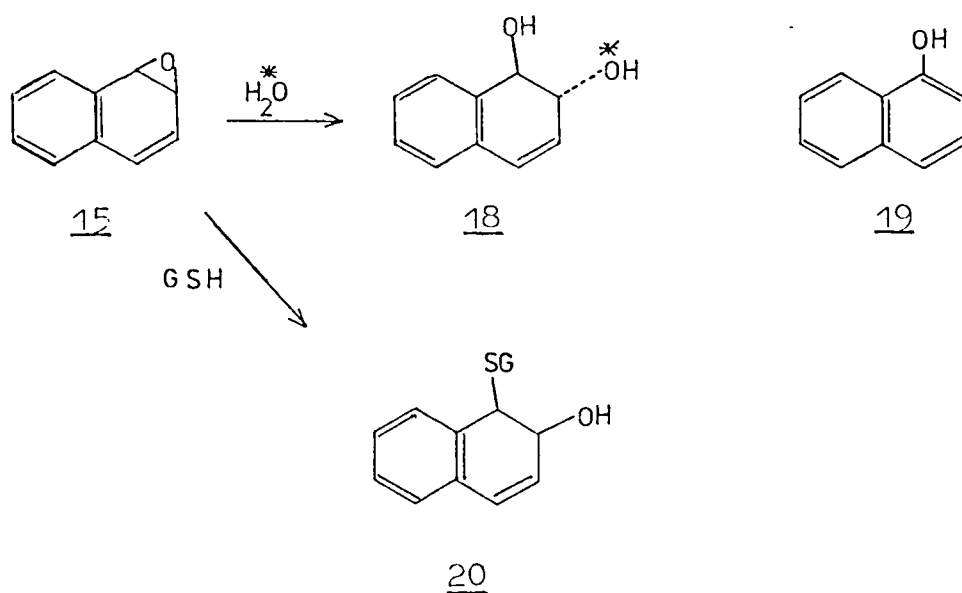
Fig. 15



Microsomal incubations of benzene did not afford benzene oxide 16, since the compound is too unstable.

The tricyclic naphthalene-1,2-oxide 15 is one of the three major products 15, 18 and 19 of the microsomal oxidation of naphthalene by solubilised liver microsomal mono-oxygenase systems ⁽¹³⁾ (Fig. 16).

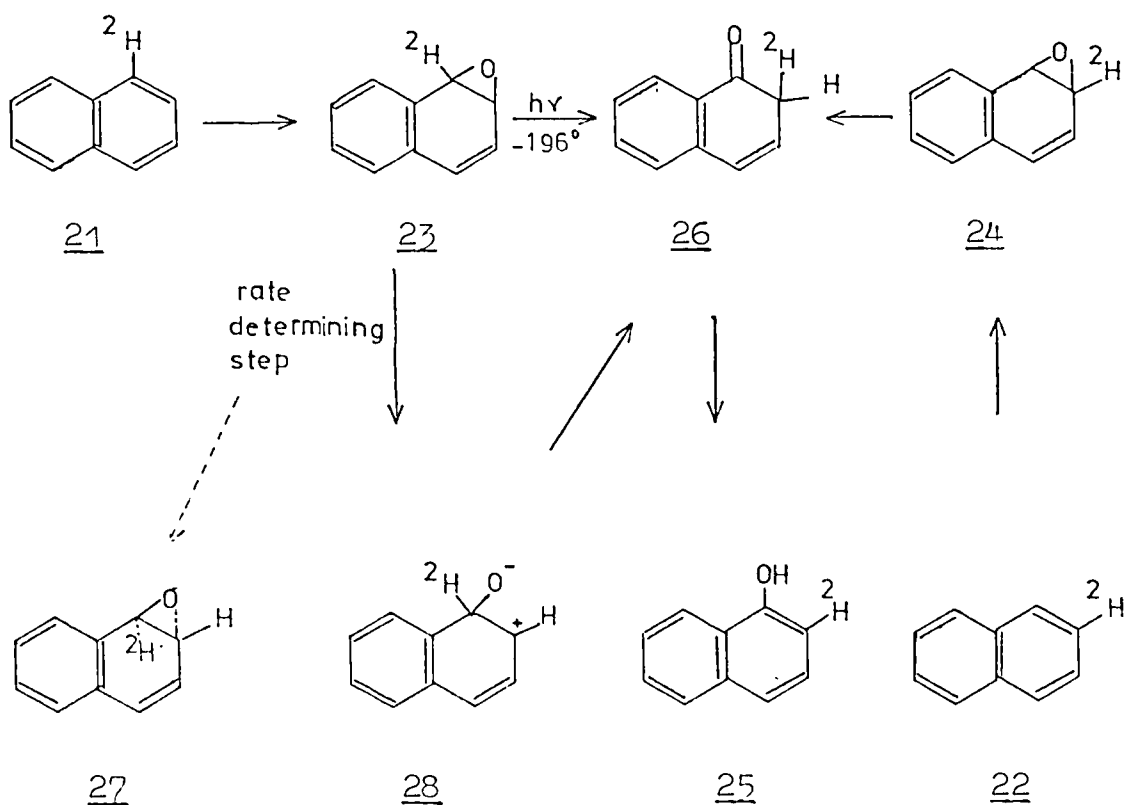
Fig. 16



The spontaneous aromatisation of 15 leads almost exclusively to α -naphthol 19 ; the stereospecific ring opening by epoxide hydrazine gives the levorotatory trans-(1R,2R)-glycol 18 ⁽¹⁵⁾ . If an excess of glutathione and glutathione transferase are present, successful nucleophilic competition with water arises and the premercapturic acid 20 is formed from the obligatory common epoxide 15 .

Studies on $[1-^2\text{H}]$ and $[2-^2\text{H}]$ naphthalene 21 and 22 , and its 1,2-oxides 23 and 24 , helped to solve several questions about the actual reaction mechanism ⁽¹⁶⁾ (Fig. 17).

Fig. 17



When the deuterated naphthalenes 21 and 22 are directly hydroxylated to α -naphthol 25 there is a NIH shift and the deuterium retentions are comparable (Table 1).

Table 1

Hydroxylation of $[1-^2\text{H}]$ and $[2-^2\text{H}]$ naphthalene with liver microsomes and two chemical model systems. The retentions are accurate to $\pm 8\%$.

Oxidant	% retention in α -naphthol	
	$[1-^2\text{H}]$ naphthalene	from $[2-^2\text{H}]$ naphthalene
microsomes	64	64
pyridine N-oxide, $h\nu^{(17)}$	68	64
m.Clpbz. acid (16)	60	68

The deuterium retentions observed in the spontaneous aromatisation of the two naphthalene oxides 23 and 24 to α -naphthol 25 are comparable under neutral or basic conditions (Table 2). This should indicate a common intermediate 26, the keto-tautomer of α -naphthol, which can be formed directly in situ at -195°C by irradiation of naphthalene 1,2-oxide. Compound 26 rearranges spontaneously and quantitatively to α -naphthol on warming to -70°C ⁽¹⁸⁾.

Table 2

Deuterium retention after isomerisation of $[1-^2\text{H}]$ and $[2-^2\text{H}]$ naphthalene oxide to 1-naphthol ⁽¹⁶⁾. The retentions are accurate to $\pm 2\%$.

Isomerisation conditions	% deuterium remaining in 1-naphthol after isomerisation	
	1-deuterium	2-deuterium
Liver microsomes (pH 9)	75	72
Acetic acid	70	83
pH 3	59	85
pH 4	58	85
pH 5.5	71	84
pH 7.0	80	81
pH 8.5	81	80

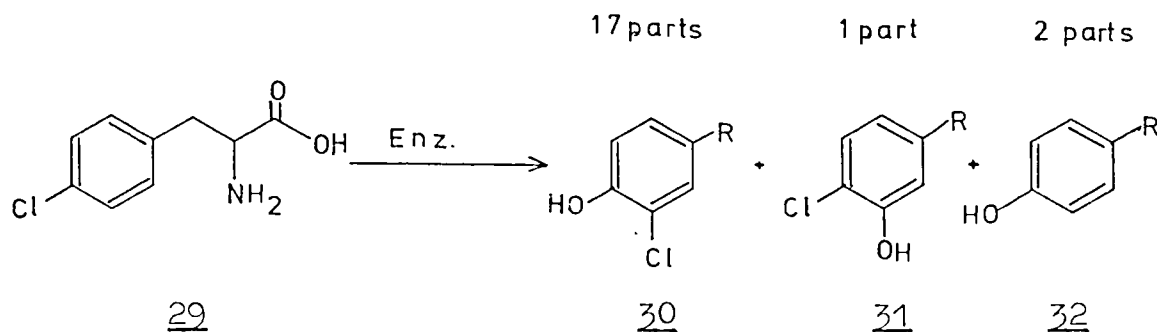
The triangular transition state 27, resulting from intramolecular hydride shift was considered and rejected, because the primary isotope effect expected from a fully deuterated arene oxide was not observed ⁽¹⁹⁾.

The rate determining step in the spontaneous rearrangement is now considered to be the development of a charge in the zwitterionic transition state 28 without ^2H

transfer. In the acid catalysed isomerisation the rate determining step is the collapse of the protonated arene oxide to the carbonium intermediate (Fig. 14).

Halogen and alkyl groups can also give the NIH shift. p-Chlorophenylalanine 29 shows migration of chlorine to the m-position (30), as well as m-hydroxylation (31) and loss of chlorine (32) ⁽²⁰⁾ (Fig. 18).

Fig. 18



4-Methylphenylalanine shows a unique migration to m-methyltyrosine by the action of phenylalanine hydroxylase ⁽²¹⁾.

The isomerisation of 8,9-indan-oxide 33 to 4-indanol 34 has been explained by assuming a circumambulatory oxygen movement (oxygen walk) , 35 $\rightarrow$ 36 $\rightarrow$ 37 $\rightarrow$ 34 and 37 $\rightarrow$ 38 $\rightarrow$ 39 $\rightarrow$ 40 , explaining the formation of both 4- and 5-indanol ⁽²²⁾ (Fig. 19).

Arene oxides are very easily opened by weak nucleophiles. Such openings may proceed by 1,2- , 1,4- and 1,6-addition of nucleophiles (Fig. 20). This was shown by using benzene oxide labelled with deuterium in the 2,5 positions 41 ⁽²³⁾; products 42 , 43 and 44 are respectively obtained.

Fig. 19

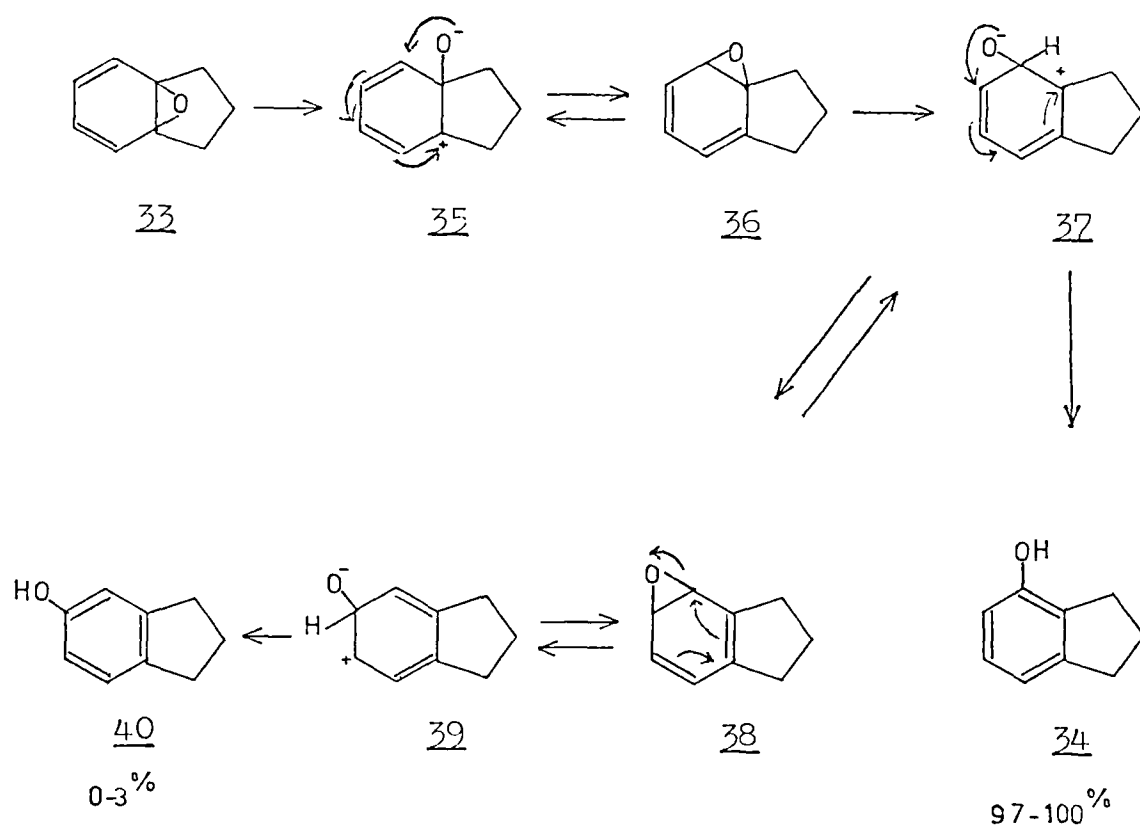
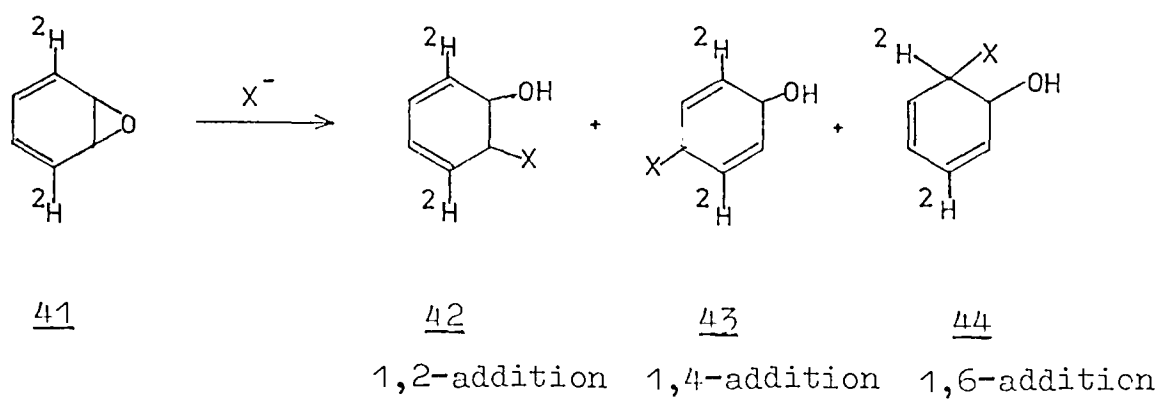


Fig. 20



3. CHEMICAL MODELS FOR AROMATIC HYDROXYLATION

The diagnostic importance of the NIH shift is remarkable : oxidation systems that do not show it are not valid models for monooxygenases. On the other hand, oxidants that show the shift must go through arene oxides in the same way as in the corresponding enzymatic hydroxylations.

Polycyclic arene oxides are often referred to as 'K-region' or 'non-K-region' oxides. This nomenclature arises from work by Pullman (24) which showed that carcinogenic hydrocarbons had regions which could be considered as having double bond character as opposed to a delocalised system ; more often than not the in vivo oxidation of these hydrocarbons occurred at such regions (K-regions).

The nature of the oxidising species generated from molecular oxygen by cytochrome P 450 is unknown. Most authors agree that an "oxene" or "oxenoid" mechanism is operating , whereby the equivalent of an oxygen atom transfer occurs during the oxidation. Similarity between the reactions of carbenes and nitrenes (addition to double bonds, C-H bond insertion) with the oxidation catalysed by cytochrome P 450 (epoxidation, arene oxide formation, aliphatic hydroxylation) have led to this oxene terminology. The range of reactions catalysed by cytochrome P 450 are effected by three general types of oxidants (25):

a) Molecular oxygen in the presence of a metal ion and a source of reducing equivalents

Such reactions are exemplified by the Udenfriend system (26,27). Fe(II), O₂ and ascorbic acid are employed.

b) The reactions of H_2O_2 and organic peroxides

Some examples are the metal catalysed decomposition of H_2O_2 (28,29) or organic peroxides (17) and the reactions of peroxy acids (30,31). In these reactions reducing equivalents are not consumed.

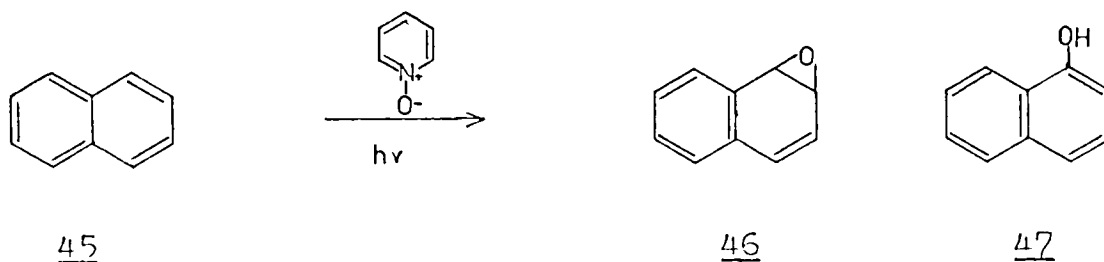
c) Non-peroxidic oxidants which transfer oxygen

These include photoexcited azaromatic N-oxides (17) and inorganic reagents such as chromyl acetate (32).

There are two main problems encountered with such model systems : the complexity of the reactions and the question of what criteria should be applied when comparing models to the enzyme-catalysed systems. The occurrence of the NIH shift has provided one invaluable criterion for such comparison. Models which do not display this phenomenon must be considered inadequate, despite how attractive they might appear from other standpoints.

The photolysis of pyridine N-oxide in the presence of naphthalene 45 gave both naphthalene-1,2-oxide 46 as well as α -naphthol 47 (17) (Fig. 21). That is why pyridine N-oxide

Fig. 21

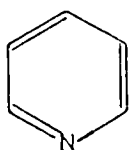


is considered to be a reasonably good biomimetic hydroxylating agent. The exact nature of the oxidising species of this biomimetic model has not yet been fully characterised and one of the aims of the current work was to investigate its real nature.

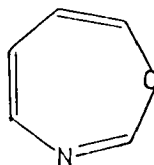
4. THE PHOTOCHEMISTRY OF AROMATIC N-OXIDES

The photochemical behaviour of aromatic N-oxides has been extensively reviewed⁽³³⁾. When aromatic N-oxides are irradiated with u.v. light several products are formed : deoxygenation products (48) , oxazepines (49), lactams (50) and 2-formyl-pyrroles (51) are amongst the main products (Fig. 22).

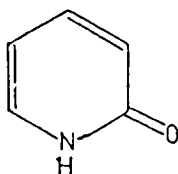
Fig. 22



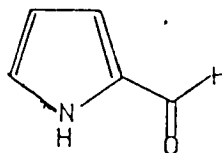
48



49



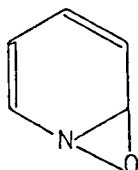
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51

Apparently two main reaction paths are followed : 1) the deoxygenation and 2) the formation of an oxaziridine 52, originating all the rearrangement products (Fig. 23).

Fig. 23

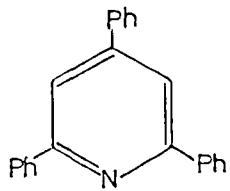
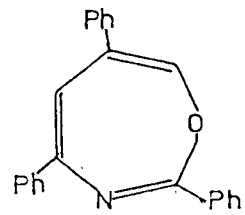
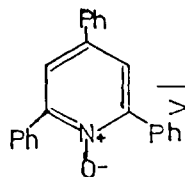
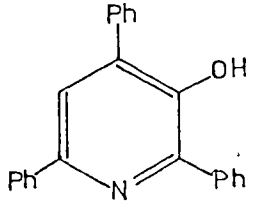
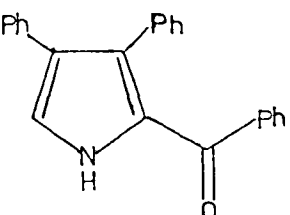
52

Such oxaziridines have never been isolated from the photolysis of heteroaromatic N-oxides. Their existence , however, gives a coherent and logical explanation for all the subsequent rearrangement products. The deoxygenation mechanism appears to take place from a triplet excited state ⁽³⁴⁾: 2,4,5-triphenylpyridine N-oxide 53 was photolysed in the absence and in the presence of benzophenone, which is known to be a triplet sensitiser. The enhanced formation of the deoxygenated amine 54 with a diminished formation of the other rearrangement products when benzophenone is used indicates that the deoxygenation takes place from an excited triplet state (Fig. 24).

The chemical properties of singlet and triplet excited states of 2-cyano-pyridine N-oxide and of pyridine N-oxide were also examined ⁽³⁵⁾. The sensitisation of the pyridine N-oxide triplet state increases the extent of the oxygen transfer reactions, whereas quenching with a copper salt in aqueous solution increases the extent of photo-rearrangement (Table 3 and Table 4).

Fig. 24

Photolysis of 2,4,5-triphenylpyridine N-oxide with a triplet sensitiser (benzophenone)

	Products	Yields	a	b	c
<u>54</u>		3 parts	21 %	8 %	only
		2 parts			none
<u>53</u>					
	$\xrightarrow[\text{> } 3000 \text{ \AA}]{h\nu}$				
		10 %	38 %	none	
		56 %	37 %	none	

a = acetone

b = methanol

c = ethanol and benzophenone

Table 3

Yield of 2-cyano-pyridine in the presence or absence of sensitisers (2×10^{-2} M solutions of 2-cyano-pyridine N-oxide in dichloromethane)

Sensitiser	Yield (%)
None	47.3
Xanthone [ET 74 Kcal mol ⁻¹]	
4×10^{-2} M	57.6
8×10^{-2} M	63.0
1.6×10^{-1} M	71.0
Tetrabutylammonium phenyl-tetrazolide	
[ET 79 Kcal mol ⁻¹]	
3×10^{-2} M	71.7
Oxygen bubbled through the solution	33.0

From Table 3 it can be seen that an increased concentration of triplet sensitiser increases the yield of deoxygenated product up to 71%. When oxygen (O_2 is a triplet quencher) is bubbled through the solution the yield of deoxygenated product drops to 33.0%.

The same authors postulate a singlet excited state for the formation of the other photoisomers. A quenching of the triplet state of pyridine N-oxide should therefore lead to an increased yield of 2-formyl-pyrrole. A transition

metal complex was thought to quench the triplet state and in fact the formation of 2-formyl-pyrrole was increased tenfold when a copper complex in water was irradiated (Table 4). However the results of Table 4 are not general,

Table 4

Irradiation of pyridine N-oxide in water in the presence of copper salts, and yield of 2-formyl-pyrrole

Reactant	Yield (%)	Time (h)
Pyridine N-oxide ($2 \cdot 10^{-2} \text{M}$)	2	100
$[\text{PyO}]_2 [\text{Cu NO}_3]_2$ ($1 \cdot 10^{-2} \text{M}$)	32	100
Pyridine N-oxide ($2 \cdot 10^{-2} \text{M}$) $\text{Cu}[\text{ClO}_4]_2$ ($2 \cdot 10^{-1} \text{M}$)	40	6

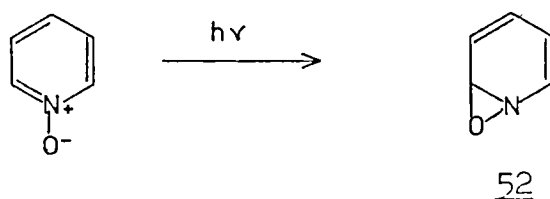
since when other metals were employed (Zn^{2+} , Fe^{3+} , Ni^{2+} and Co^{2+}) no increase in the formation of 2-formyl-pyrrole could be observed.

These experiments also support the existence of two different photochemical mechanisms : the deoxygenation process, from a triplet excited state, and the photo-rearrangements, possibly from a singlet excited state.

The photorearrangement

All the rearrangement products obtained by irradiation of aromatic N-oxides can be explained by assuming the initial formation of an oxaziridine intermediate 52 (Fig. 25).

Fig. 25

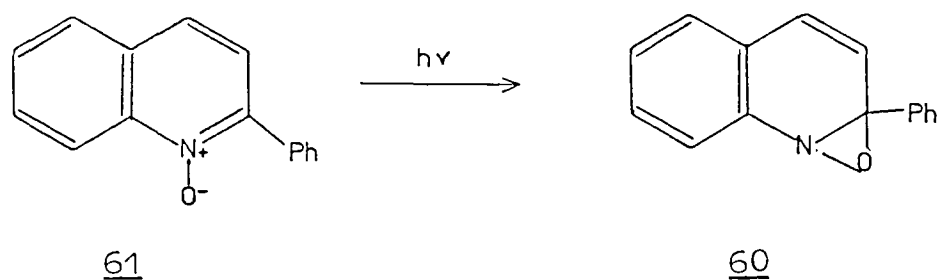


There is no direct chemical proof for this assumption which was postulated from the known behaviour of nitrones 55 , which are known to give oxaziridines 56 under photochemical conditions (36) . The same oxaziridine could be obtained by treatment of the corresponding imine 57 with peracid. The intermediate oxaziridine rearranges to the amides 58 and 59 (Fig. 26).

A Japanese group (37) thought they had actually isolated the oxaziridine 60 from 2-phenylquinoline N-oxide 61 (Fig. 27).

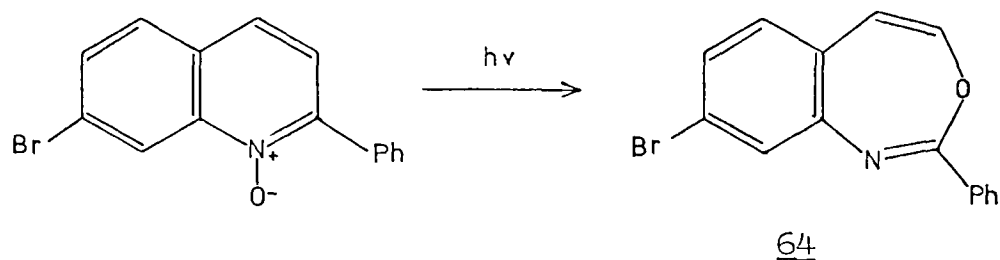
Buchardt, Djerassi and coworkers (38) disproved it, showing how the compound obtained by Kaneko and coworkers had a different structure 64 from the one proposed by the Japanese chemists (Fig. 28). Compounds 62 have been assigned a benz[f]-1,3-oxazepine structure, rather than the oxaziridine structure 63 . U.v., i.r., n.m.r. and mass spectral data, as well as chemical evidence, support the

Fig. 27



seven-member ring structure 62 . The oxazepine structure 64 was also confirmed by an X-ray determination ⁽³⁹⁾(Fig. 29).

Fig. 29



To date , the oxaziridine intermediates have never been isolated from the product mixtures from the photolysis of aromatic N-oxides. There are four possible reasons for this :

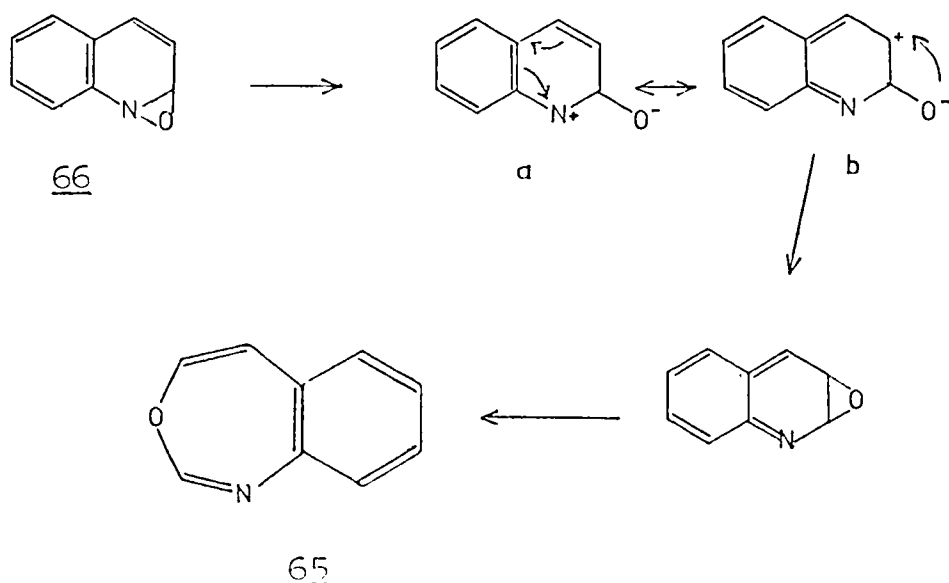
i) The oxaziridines of aromatic N-oxides are thermally unstable. Perhaps if less energetic light is used , and very low reaction temperatures are employed, these intermediates can be discovered spectroscopically. Buchardt ⁽⁴⁰⁾ irradiated some 2-phenylquinoline N-oxide at -190°C in an ethanol glass : the colourless solution turned red. The reaction mixture turned yellow upon heating (recooling did not influence this colour) and a thin layer chromato-

graphic analysis showed that the expected benz[d]-1,3-oxazepine was formed. This experiment clearly indicates the existence of a thermally unstable species, which might be the oxaziridine.

- ii) Excited atomic rearrangements, corresponding to oxaziridines, may be on the reaction pathway, but only as transition states or species with a very short lifetime.
- iii) Oxaziridines may be formed, but break down photochemically with a quantum yield equal to, or greater than, that of their formation.
- iv) Oxaziridines are not formed as intermediates and an entirely different mechanism is operating.

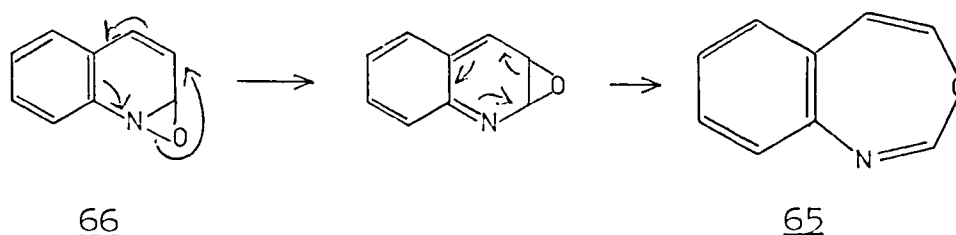
According to Buchardt, the formation of the oxazepine 65 should take place from the oxaziridine 66 ⁽⁴¹⁾. The three membered ring opens heterolytically to form an epoxide ring, which can then rearrange to the seven membered ring (Fig. 30).

Fig. 30



The same author does not rule out the possibility of a thermal 1,5-sigmatropic shift (symmetry-allowed) (Fig. 31).

Fig. 31



Formation of lactams

One of the more salient features in the photolysis of the quinoline N-oxide series is the solvent effect. Polar protic solvents favour carbostyryl formation , whereas non-polar aprotic solvents favour benz [d]-1,3-oxazepine formation. A mechanistic interpretation in agreement with this observed solvent effect is the mechanism showed in Fig. 32 .

In polar protic media the zwitterionic species , and the transition states leading to those, are expected to be energetically more favoured than in non-polar protic media.

Buchardt ⁽⁴²⁾ photolysed [2-²H]-quinoline N-oxide 67 : according to the scheme shown in Fig. 33 a C-N shift or a C-C shift would give different products. [3-²H]-Carbostyryl 68 could be found : [1-²H]-carbostyryl 69 readily exchanges with the solvent. Buchardt proved how both mechanisms operate and that the C-C hydrogen shift

Fig. 32

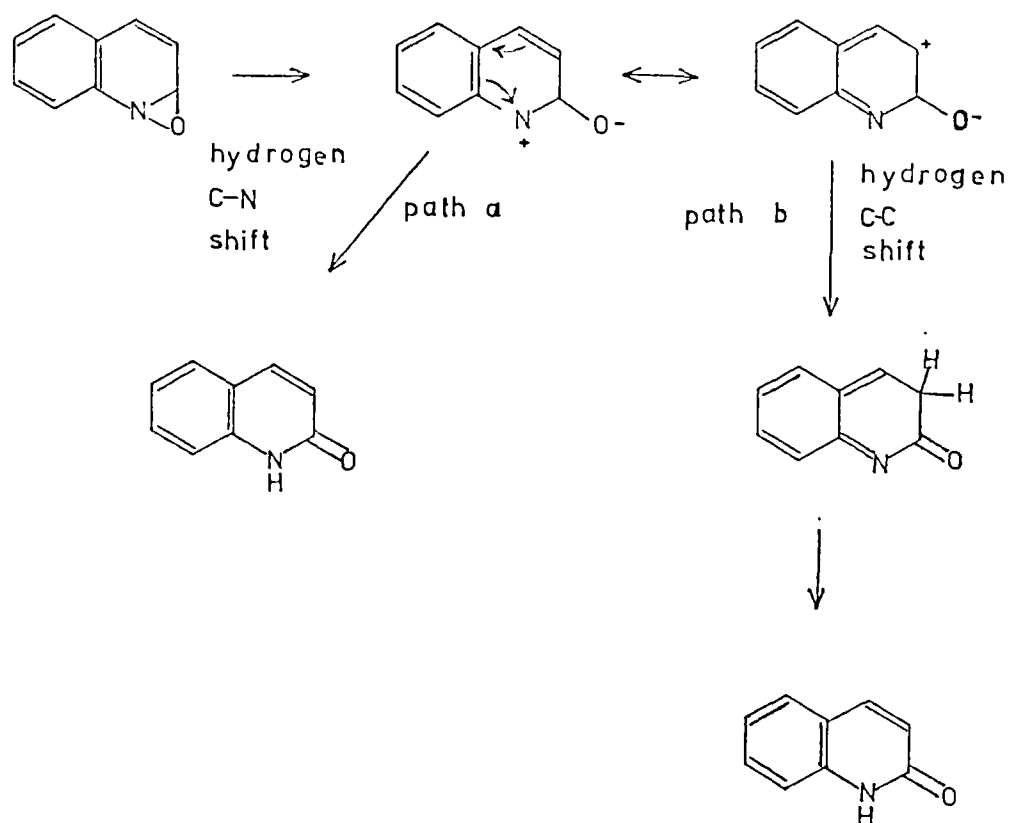
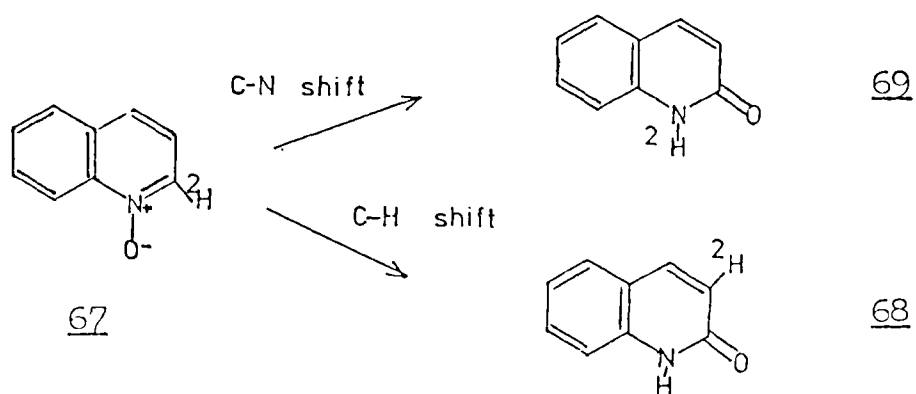
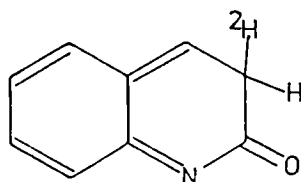


Fig. 33



is favoured in polar solvents. The isotope ratio (D/H) observed at the 3-position for the carbostyryl is 2.11 for water, 1.1 for 95% ethanol, 0.97 for absolute ethanol, 0.73 for benzene and 0.58 for ether. The isotope effect for loss of hydrogen from 70 (Fig. 34) would lead to $D/H = 3.5$ (which is probably a maximum ⁽⁴³⁾). The D/H ratio in water indicates that at least 85% of the carbostyryl is formed via the C-C 1,2-hydrogen shift in water, and ca 63% in ethanol. In aprotic solvents such as benzene or ether the proportion formed by it drops to 55% and 45% respectively.

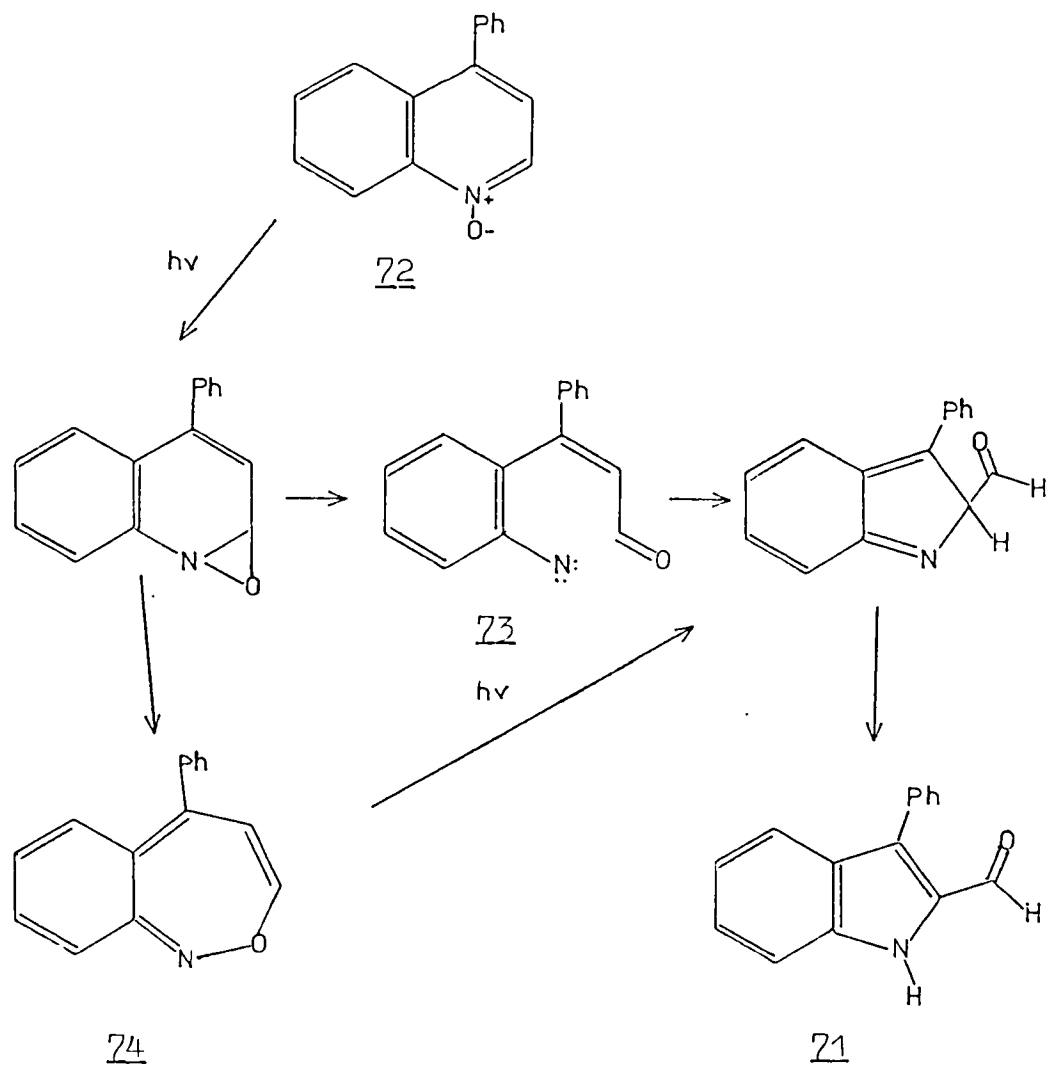
Fig. 34

70

Formation of 2-pyrrole-aldehyde type compounds

The formation of 3-phenyl-2-indolecarboxaldehyde 71 in the photolysis of 4-phenylquinoline N-oxide 72 is depicted as taking place via a nitrene 73 ⁽⁴⁴⁾. This ring contraction could also be explained by assuming formation of the benzooxazepine 74 which could undergo a light induced 1,3-sigmatropic shift (Fig. 35).

Fig. 35



5. THE DEOXYGENATION AND CONCOMITANT FORMATION OF OXIDATION PRODUCTS FROM HETEROAROMATIC N-OXIDES

Hata and Tanaka ⁽⁴⁵⁾ studied the light induced reaction of pyridine N-oxide in the gas phase, using either 3261 or 2537 Å light. In each case the main product was

pyridine. Similarly vapour phase photolysis of 3-methylpyridine N-oxide was reported to give mainly the parent 3-methylpyridine (46).

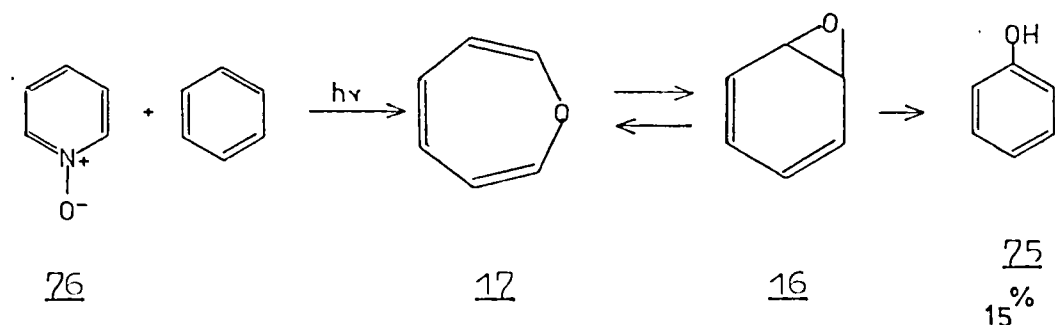
A unique result is the finding by Hata (47) that irradiation of 2-methylpyridine N-oxide in the vapour phase with 3261 Å light resulted in the formation of 2-hydroxymethylpyridine, whereas irradiation with 2537 Å light resulted in deoxygenation.

An extremely important paper appeared in 1967 (48) and it reports the formation of phenol 75 on irradiating pyridine N-oxide 76 in benzene. The formation of atomic oxygen at room temperature is postulated and a 15% yield of phenol was obtained. According to the authors, the formation of oxepine 17, the more abundant valence tautomer of benzene oxide 16 (49), could have been expected but no choice could be made between the addition reaction or the insertion of oxygen into a C-H bond, since the oxepine is known to rearrange to phenol under very mild conditions, including with bases (such as collidine). The proposed scheme is shown in Fig. 36.

The yields quoted by the authors were in our hands irreproducible ($\approx 1.0\%$ phenol instead of 15%) and the mechanism proposed by the authors does not appear extremely convincing. The work made by Jerina and coworkers is in agreement with our yields of hydroxylated products and also gives convincing explanations on the reaction mechanism. Furthermore the experimental details of this important reaction are not mentioned at all. All these

remarks do not affect one important conclusion : the photolytic deoxygenation of aromatic N-oxides takes place with the concomitant release of an oxidising species. The postulation of "atomic oxygen" generated at room temperature appears attractive as well, but no further work from the authors on this interesting topic has ever been made.

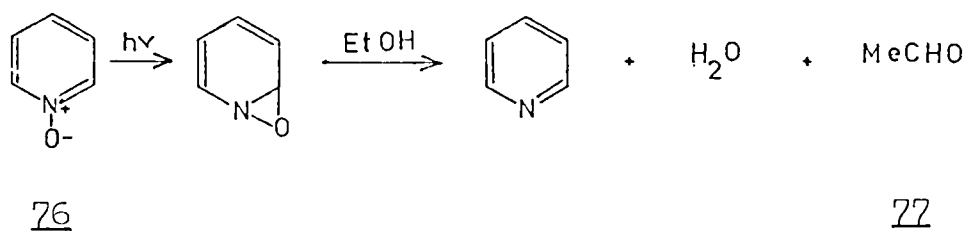
Fig. 36



Alkaitis and Calvin ⁽⁵⁰⁾ observed the formation of acetaldehyde 77 on irradiating pyridine N-oxide 76 in ethanol. They attributed the formation of the oxidation product to the intermediate formation of the oxaziridine, which is known to act as an oxidising agent. An oxaziridine oxidises iodide to iodine ⁽⁵¹⁾. The parent imine is formed as a product. The scheme invoked is shown in Fig. 37 .

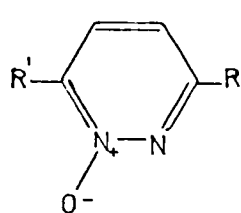
The photo-induced oxygenation of hydrocarbons by pyridazine N-oxides has been studied by Igeta and coworkers ⁽⁵²⁾. The photolysis of the pyridazine N-oxides 78 and 79 (Fig. 38) in benzene solution resulted in the formation of the

Fig. 37



corresponding pyridazines and phenol as the main products with moderate yields (30-40%). The same products were

Fig. 38



78 $R = R' = \text{H}$

79 $R = \text{H} ; R = \text{CH}_3$

obtained when these N-oxides and benzene (5-10 mole equivalent to the N-oxide used) were irradiated in dichloromethane. When 78 and 79 were irradiated in toluene a mixture of cresols was obtained in about 35-40% yield and no benzyl alcohol was isolated. Naphthalene (5-10 mole equivalent) was irradiated in dichloromethane with these N-oxides and a 20-30% yield of naphthols was obtained (1:0.8 ratio of $\alpha:\beta$).

Aliphatic compounds could also be oxygenated by this procedure. Pyridazine 1-oxide 78 was irradiated in dichloromethane, with a 5-10 molar excess of cyclohexane

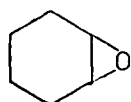
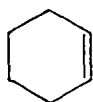
and a 30% yield in cyclohexanol was obtained. When an equimolar mixture of cyclohexane and benzene was photolysed in dichloromethane, using a 5 molar excess of each component with respect to pyridazine 1-oxide 78, the amount of phenol formed was about twice as much as that of cyclohexanol. The reaction products were identified by both gas-chromatography and the formation of crystalline derivatives.

The photo-induced oxidations of ethylenic compounds were studied by the same group ⁽⁵³⁾. Various pyridazine N-oxides were used and similar results were obtained in each case ; 6-methylpyridazine 1-oxide, pyridazine 1-oxide 78, 3-methylpyridazine 1-oxide 79 and 3,6-dichloropyridazine 1-oxide were tried. The results are summarised in Fig. 39. A mixture of 6-methylpyridazine 1-oxide and 2-3 mole equivalents of olefinic compounds were irradiated in dichloromethane. The high pressure mercury lamp was used, with a Pyrex filter, under a nitrogen atmosphere, until all the N-oxide was consumed. The total yields vary from 15 to 25%. When either styrene 80 or indene 81 were irradiated under these conditions the oxygenation products of the ethylenic bond predominated over those of the aromatic ring. The oxidising species appears to have a certain degree of stereoselectivity : cholesterol 82 was oxygenated to give mainly the α -epoxide 83 (α : β epoxide ratio = 8:1). 3 β -Cholesteryl acetate 84 does not show the same degree of selectivity, although the α -epoxide 85 still predominates : a 5:3 ratio of the α - to the β -epoxide 86 is obtained. One interesting feature in this product distribution is its

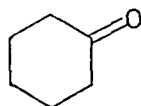
Fig. 39

Ethylenic compounds

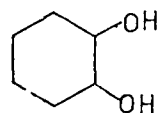
Products



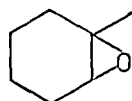
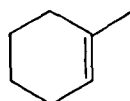
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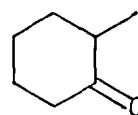
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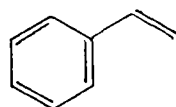
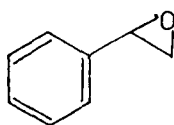
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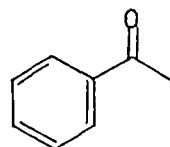
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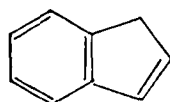
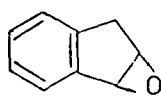
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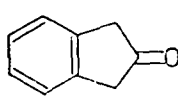
15 parts



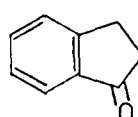
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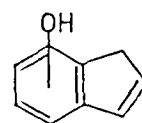
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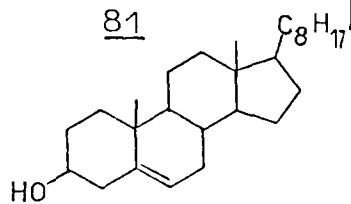
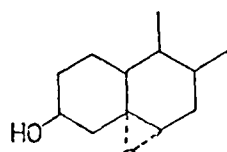
4 parts



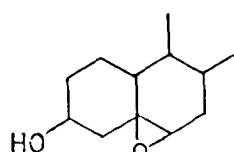
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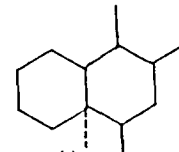
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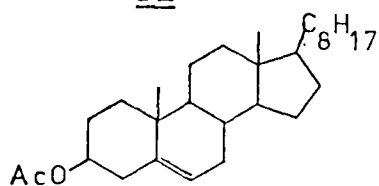
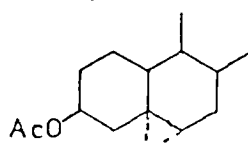
8 parts



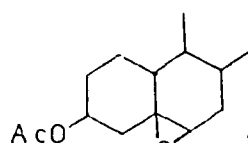
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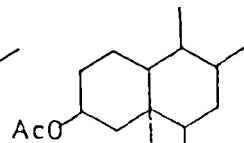
3 parts

8485

5 parts

86

3 parts



1 part

similarity to the one obtained from the perbenzoic acid oxidation of these materials ⁽⁵⁴⁾.

One important feature of the deoxygenation of pyridazine N-oxide is the relatively high deoxygenation yield ; by comparison pyridine N-oxides normally give only low amounts of deoxygenation (below 10%) ⁽³³⁾. Igeta and coworkers postulate an oxygen atom addition to the aromatic ring to form oxepines, which then rearrange to phenols . They seem to accept the mechanism proposed by Streith and coworkers ⁽⁴⁸⁾ for the formation of phenol from benzene and pyridine N-oxide. The most thorough work on this type of aromatic hydroxylation has been reported by Jerina, Daly, Witkop and coworkers at the National Institutes of Health, Bethesda, Maryland. The group was elaborating chemical oxidants which exhibit the NIH shift and form arene oxides in aromatic hydroxylation reactions. Their objective was to define the chemical nature of the active oxygen involved in enzymatic hydroxylations. Pyridazine N-oxide, pyridine N-oxide and pyrazine N-oxide were used in the hydroxylation of $[4-^2\text{H}]$ -anisole. This particular substrate was chosen since there is a high migration and retention (60%) of deuterium under microsomal hydroxylation ⁽¹¹⁾. The results obtained are shown in Table 5 . All the irradiations were carried out in quartz cells, using 2537 Å light for 10 to 20 minutes, under nitrogen.

The substituent effect on the degree of deuterium retention during pyridine N-oxide photolysis was similar to the effect observed with microsomes (Table 6).

Table 5

Oxidant	% deuterium retention in 4-hydroxyanisole
Pyridazine N-oxide	34
Pyridine N-oxide	45
Pyrazine N-oxide	52

Table 6

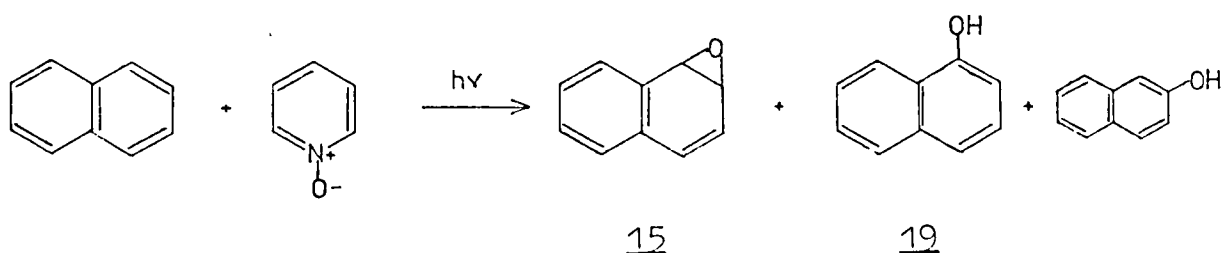
Comparison of % deuterium retention with different substrates and pyridine N-oxide

[4- ² H] substrate	% retention in 4-hydroxylated product	
	Microsomes	N-oxide irradiation
Anisole	60	45 (25° C) 60 (-78° C)
Chlorobenzene	54	62
Bromobenzene	40	49
Acetanilide	30	28
Toluene	54	59

Dichloromethane was used as solvent and the temperature was 25° C. With toluene, the substrate was the solvent.

These results strongly suggested the intermediacy of arene oxides in the hydroxylation of aromatic substrates with pyridine N-oxides. With naphthalene and pyridine N-oxide the actual 1,2-epoxynaphthalene 15 (1% yield) was isolated together with the naphthols (1% yield ; 95:5 α : β ratio) ⁽¹⁷⁾ (Fig. 40).

Fig. 40



This very important reaction constitutes the first example of chemical epoxidation of an aromatic "double bond". Under photolytic conditions the same group ⁽¹⁶⁾ found a very similar deuterium retention in the product α -naphthol by photolysing either $[1-^2\text{H}]$ -naphthalene 21 or $[2-^2\text{H}]$ -naphthalene 22 with pyridine N-oxide (Table 7). The deuterium retentions observed in the spontaneous aromatization of the two labelled naphthalene oxides are practically identical under basic or neutral conditions (Table 8). These data suggest the intermediate 26 (Fig. 41), which is the keto-tautomer of α -naphthol. Structure 26 has been demonstrated in situ at -196°C by irradiation of naphthalene 1,2-oxide ; it rearranges spontaneously and quantitatively to α -naphthol on warming to the temperature of dry

Table 7

Hydroxylation of $[1-^2\text{H}]$ and $[2-^2\text{H}]$ -naphthalene with liver microsomes and pyridine N-oxide irradiation. The retentions are accurate to $\pm 8\%$.

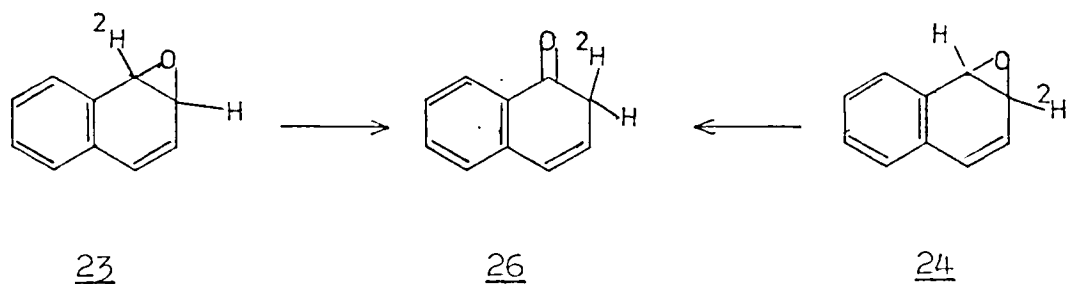
Oxidant	% retention in α -naphthol	
	from $[1-^2\text{H}]$ - -naphthalene	$[2-^2\text{H}]$ - -naphthalene
Microsomes	64	64
Pyridine N-oxide, $h\nu$	68	68

Table 8

Deuterium retention after isomerisation of $[1-^2\text{H}]$ and $[2-^2\text{H}]$ -naphthalene oxide to α -naphthol. The retentions are accurate to $\pm 2\%$.

Isomerisation conditions	% deuterium remaining in α -naphthol after isomerisation	
	$[1-^2\text{H}]$ - 	$[2-^2\text{H}]$ - 
Liver microsomes (pH 9 , tris buffer)	75	72
pH 7	80	81
pH 8.5	81	80

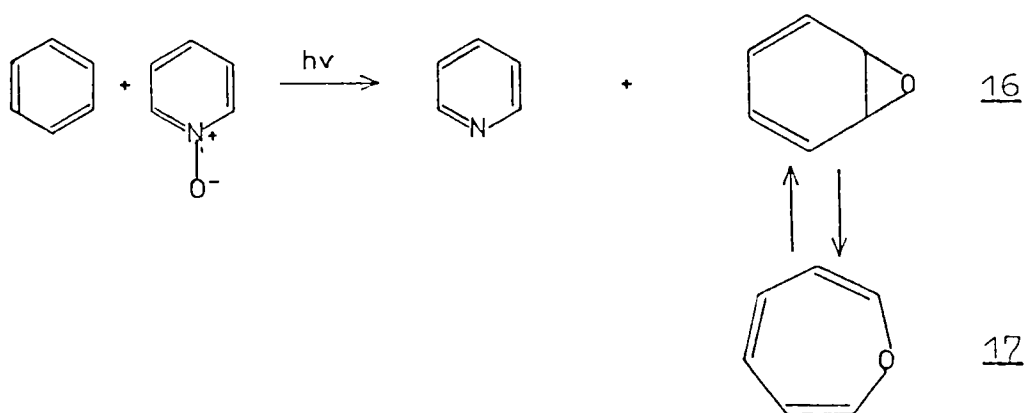
Fig. 41



ice (ca -70°C) (18). Presumably a mechanism of this type also operates in the photolysis of naphthalene with pyridine N-oxide.

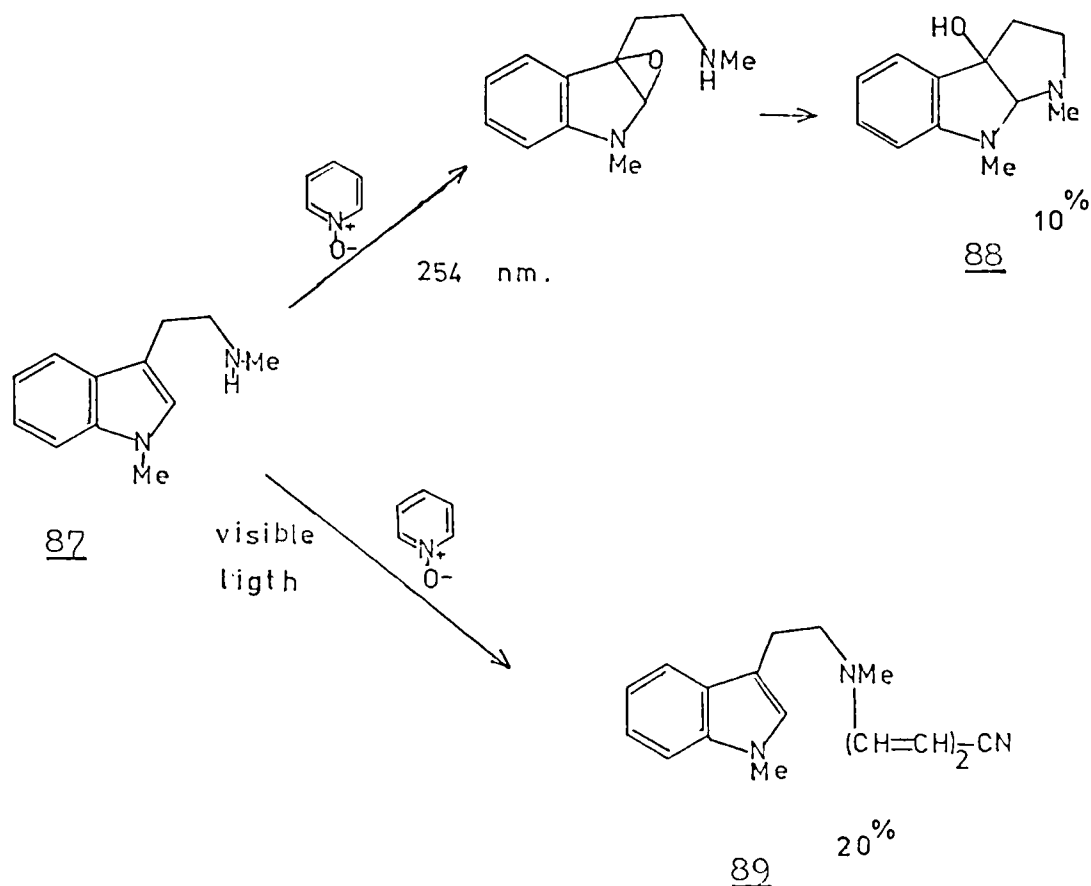
The nature of the oxidising species is not still clear, yet the mechanism proposed is the more convincing and is confirmed by the massive experimental data of Jerina and his coworkers. It appears attractive to postulate the formation of atomic oxygen ; again the experimental data are not against this hypothesis. The 'oxene' should be a powerful electrophilic species ; it could readily attack the aromatic ring and form an arene oxide 16 (Fig. 42).

Fig. 42



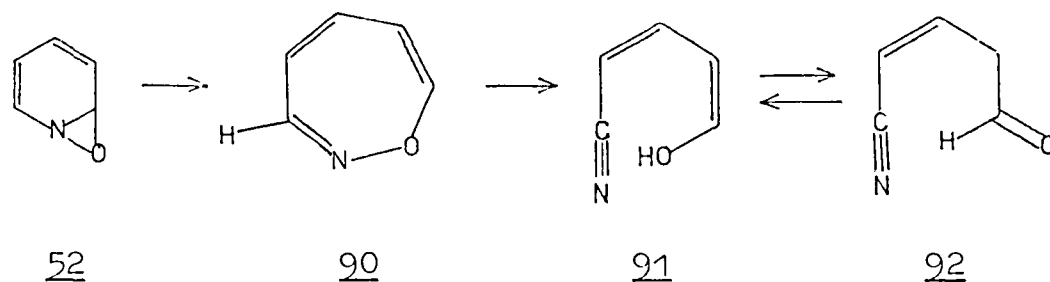
An interesting result has been obtained by Nagakawa and coworkers (55). On irradiating N^a, N^b -dimethyltryptamine 87 and pyridine N-oxide (254 nm), 1,8-dimethyl-3a-hydroxy-2,3,3a,8a-tetrahydropyrrolo[2,3-b]indole 88 was obtained. On visible light irradiation N^b -(4-cyanobutadienyl)- N^a, N^b -dimethyltryptamine 89 was obtained (Fig. 43).

Fig. 43



According to the authors only traces of 88 are obtained when visible light is used. The proposed mechanism for the formation of 89 is interesting and is summarised in Fig. 44.

Fig. 44



Pyridine N-oxide should form the oxaziridine which rearranges to the oxazepine 90. A base catalysed rearrangement, promoted by $\text{N}^{\text{a}}, \text{N}^{\text{b}}$ -dimethyltryptamine should open the oxazepine ring and the unsaturated nitrile 91 should be formed. A condensation between the α, β -unsaturated nitrile aldehyde 92 and $\text{N}^{\text{a}}, \text{N}^{\text{b}}$ -dimethyltryptamine should afford the final product 89.

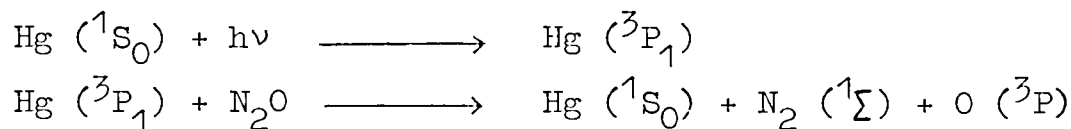
The yields of oxidation products given by Japanese and French groups are usually higher than those obtained by Jerina and coworkers. Personal opinions and comments should perhaps be avoided, but as it will appear from the later discussion of our own results, high yields, or even moderate ones, in the photochemical oxidation reactions with pyridine N-oxides should be considered with a certain amount of diffidence.

6. SOME ATOMIC OXYGEN REACTIONS

Some reactions of atomic oxygen have been reported in the literature.

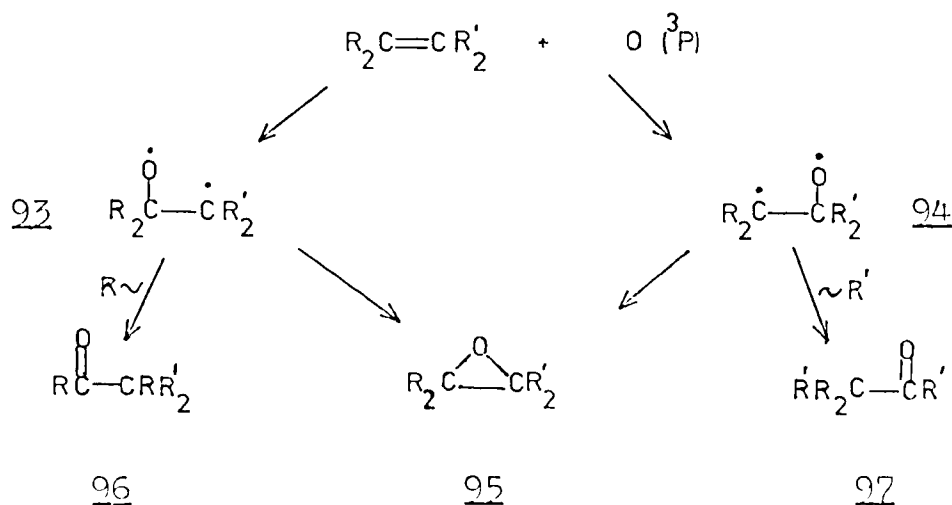
Ground state (^3P) oxygen atoms arise from the mercury photosensitised decomposition of nitrous oxide (56) (Fig. 45).

Fig. 45



The reaction of the triplet atom species with olefinic compounds has been examined by Cvetanovic (57). Ground state (^3P) oxygen is a biradical and its reactions with olefins are shown in Fig. 46 . Initially short lived

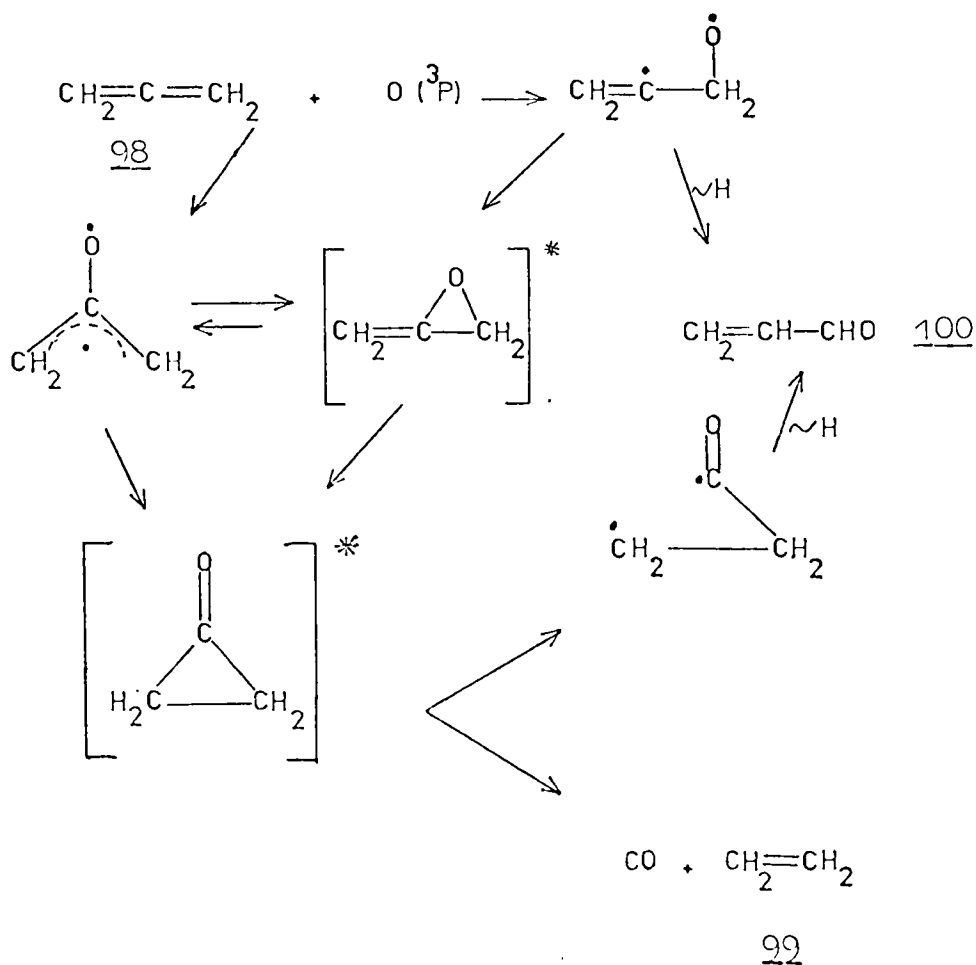
Fig. 46



1,3 biradicals 93 and 94 are formed ; such species can either close to epoxides 95 or arrange to aldehydes or ketones 96 and 97 via a 1,2 hydrogen or alkyl migration.

Havel (58) studied the reaction of $O(^3P)$ with allenes. Ground state triplet oxygen should behave as an epoxidising species that can be generated in the absence of acid. Havel's explanation of the reactions of $O(^3P)$ with allene 98 are summarised in Fig. 47 . Carbon monoxide (57% yield), ethylene 99 (38% yield) and acrolein 100 (1.1% yield) are obtained.

Fig. 47



Cvetanovic (59) obtained phenol from benzene (ca 13% of the reacted benzene is transformed into phenol). The reaction of O (^3P) atoms with toluene has been investigated by Pitts and coworkers (60). It is interesting comparing the results obtained by this group and by other separate groups (61,62); there is a general agreement on the products obtained, but there are differences in the relative amounts of the individual phenolic compounds observed. Grovenstein and Mosher (62)(c) found more m-cresol than either Jones and Cvetanovic (61)(b) or Gaffney, Atkinson and Pitts (60)(a) (Table 9).

Extensive tars are formed and there is lack of aromaticity in the tars.

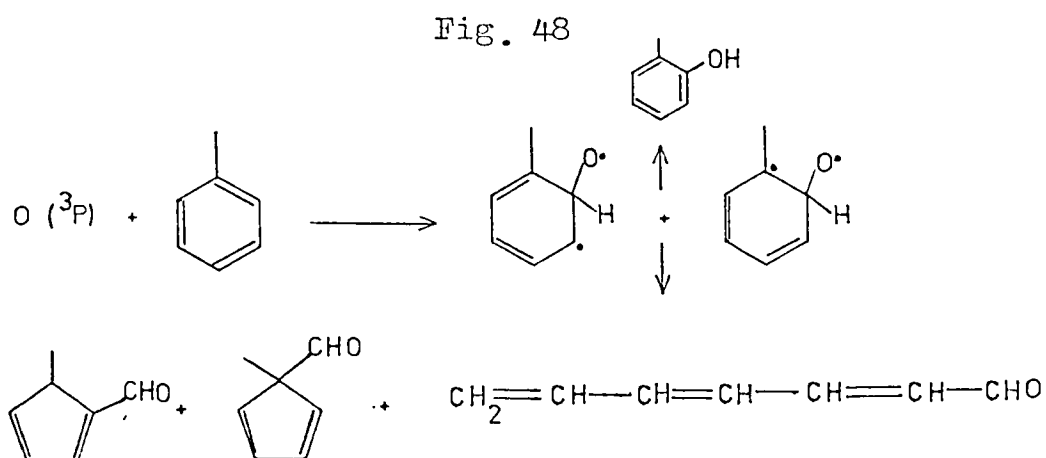
Table 9

Products observed from the reaction of O (^3P) atoms with toluene

	Yields		
	a	b	c
Phenol	8.7±1.9		8.0
<u>o</u> -Cresol	80.5±1.7	78	58.8
<u>m</u> -Cresol	4.7±0.7d	22e	15.3
<u>p</u> -Cresol			17.9

d : the m- and p-cresols were not separated ; the mass spectrum showed m-cresol 10% of the p-cresol. e : the m- and p-cresols were not separated ; the i.r. spectrum showed m-cresol 4.5% of total cresol yield.

Pitts suggested as an initial reaction step the addition of an oxygen atom to the aromatic ring, predominantly in the o-position, followed by isomerisation of phenolic compounds, ring contractions or cleavage, to form a variety of highly reactive olefinic species which react further. A summary is shown in Fig. 48 .



+ a variety of other monoaromatic unsaturated hydrocarbons

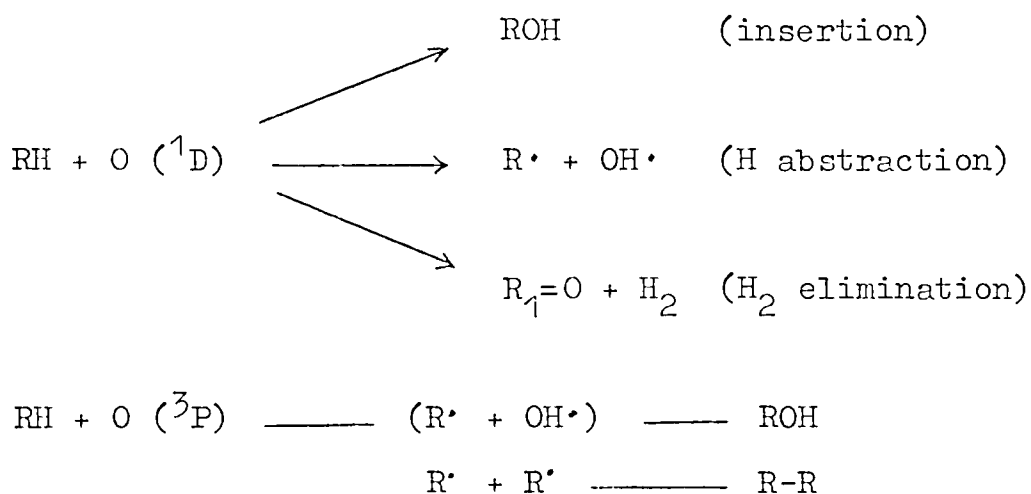
The formation of phenol presumably proceeds by addition of an $O(^3P)$ atom at the 1-position, followed by an intermolecular methyl migration and subsequent H-atom abstraction by the methyl radical to form CH_4 and by the phenoxy radical to form phenol.

The ^2H -retention in 4-hydroxyanisole obtained by N_2O mercury sensitised photooxidation of $[4-^2H]$ -anisole is not as good as with pyridine N-oxide ; a 25% retention could be observed ⁽¹⁷⁾. Low degree of ^2H retention during an aromatic hydroxylation reaction usually indicates a radical pathway and this would be

indeed the case with a $O(^3P)$ biradical generated by mercury sensitised decomposition of N_2O .

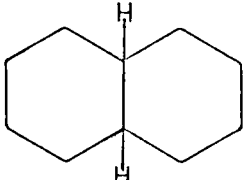
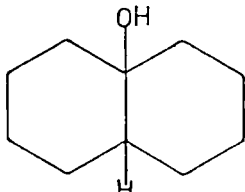
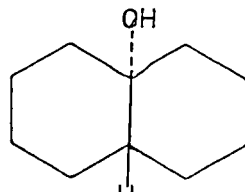
The behaviour of atomic oxygen has been recently examined by Mazur and coworkers ⁽⁶³⁾. They irradiated solutions of N_2O in saturated hydrocarbons with a low pressure mercury immersion lamp (189 nm) and the principal reaction products were alcohols, formed in almost statistical distribution ratio. Mazur assumed that the prevalent reacting species was $O(^1D)$, since this highly energetic species is known to react indiscriminately in the gas phase with all the C-H bonds in a molecule ⁽⁶⁴⁾. The small preference for the more substituted C-H bond was ascribed to the concurrent reaction of $O(^3P)$, present in smaller amounts in the solution (Fig. 49).

Fig. 49



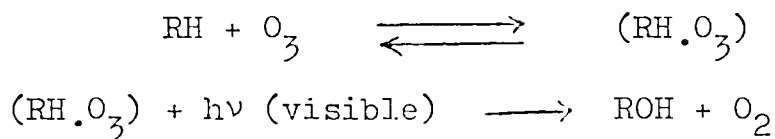
An important result was obtained with cis--decalin 101 (which is less stable than the trans--isomer). The major tertiary alcohol produced was cis-9--decalol 102, i.e. retention of configuration was observed. The $O(^3P)$ species is more regioselective in its reactivity; on diluting the cis--decalin 101 in another hydrocarbon, such as pentane, it was found that more of the tertiary alcohol was formed : $O(^1D)$ atoms react indiscriminately with all the C-H bonds, while $O(^3P)$ atoms react preferentially with tertiary C-H bonds - as the total concentration of oxygen remained unchanged, the proportion of the C-H bonds attacked by $O(^3P)$ atoms was increased (Fig. 50).

Fig. 50

$N_2O \xrightarrow{h\nu} O(^3P) + O(^1D)$		
 <u>101</u>	 <u>102</u>	 <u>103</u>
Temperature ($^{\circ}C$)	sec.:tert.	cis:trans
-15	1:1.94	96:4
0	1:1.89	95:5
50	1:1.68	93:7
-15 (10% sol.)	1:3.75	76:24

The same group examined some reactions involving the formation of atomic oxygen through ozone (65). Ozone absorbs in u.v. (λ_{max} 255 nm) and visible (λ_{max} 600 nm) spectral regions. The photolysis of O_3 at $\lambda < 302$ nm produces $\text{O} (^1\text{D})$, whilst at $\lambda > 334$ nm $\text{O} (^3\text{P})$ is formed. The reaction of O_3 with u.v. light leads to products derived from both the rearrangement of the complexes $(\text{RH}.\text{O}_3)$ and the attack of $\text{O} (^1\text{D})$ on the hydrocarbon, whilst its reaction with visible light results in products exclusively derived from the rearrangements of the complexes $(\text{RH}.\text{O}_3)$. Ozone can complex with aliphatic hydrocarbons, forming charge transfer species. These are sensitive to photochemical reaction with visible light and subsequent insertion of an atom of oxygen into the hydrocarbon takes place, with liberation of oxygen (Fig. 51).

Fig. 51



Reactions under these conditions (visible light) lead to a far greater regio and stereoselectivity than the reactions of the "free" atomic oxygen. Ozone in the charge transfer complex state therefore acts as a source of moderated atomic oxygen, that is a source of atomic oxygen (triplet ?), which is never completely free. The results obtained with cis-decalin 101 are summarised in Table 10 .

Table 10

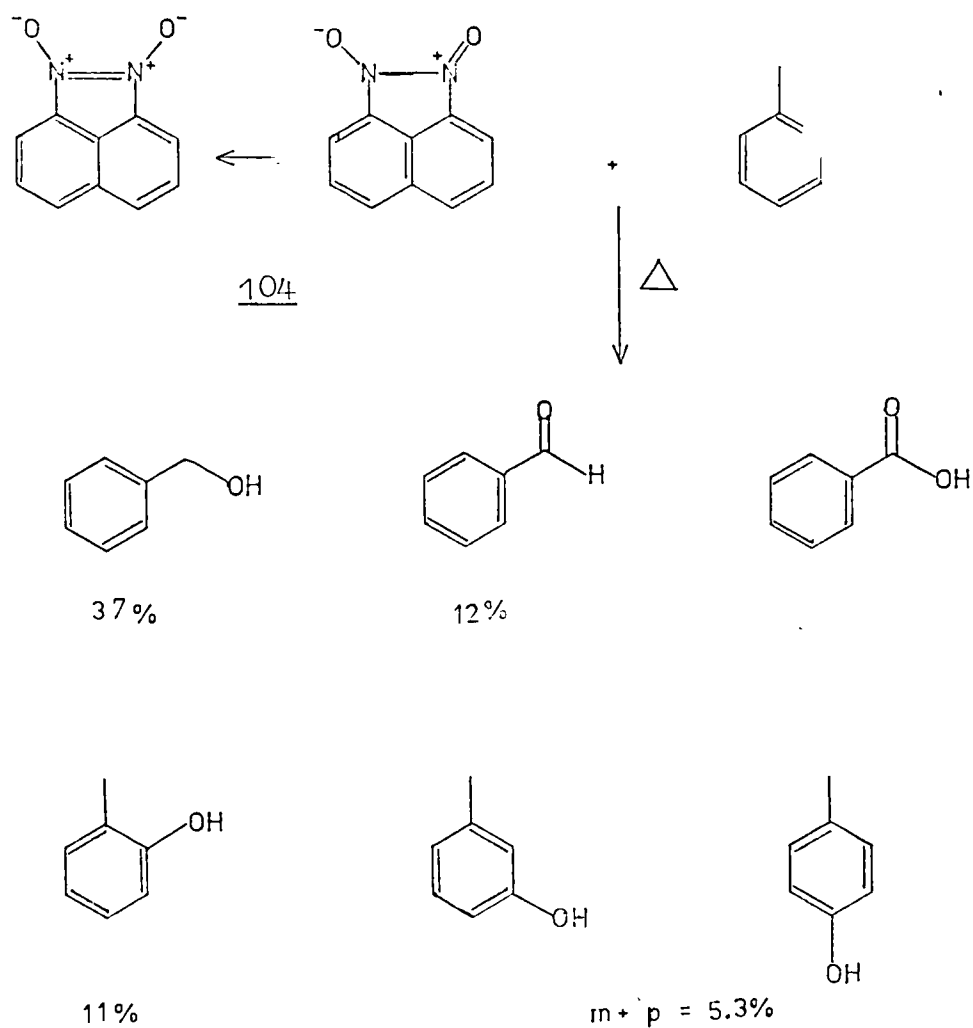
Oxidation of cis-decalin at -78°C

Source of O_2	sec.:tert.	cis:trans
O_3 (visible)	1:52.5	93.4:6.6
O_3 (254 nm)	1:8.9	72.3:27.7
N_2O (185 nm)	1:3.9	64.0:36.0

7. A THERMAL OXYGEN ATOM TRANSFER MODEL.

Kirby and coworkers (66) have recently found another oxygen-transfer model. The thermolysis of benz[c,d]-indazole 1,2-dioxide 104 at $105-110^{\circ}\text{C}$ in the presence of various benzenoid derivatives gave benz[c,d]-indazole 1-oxide and oxidation products of the benzenoid substrates. The results obtained with toluene are summarised in Fig. 52 (gas chromatographic yields). The dioxide 104 does not appear to lose both oxygen atoms under thermal conditions ; prolonged heating of the monooxide in toluene does not produce significant amounts of toluene oxidation products. The gas chromatographic yields in these reactions were checked with isotopic labelling experiments, which also provided useful informations as far as the NIH shift was concerned [$4-^3\text{H}$, methyl- ^{14}C]-toluene was oxidised with 104 and the g.c. yields

Fig. 52

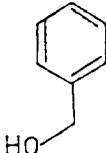
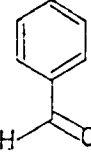
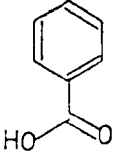
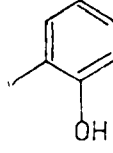
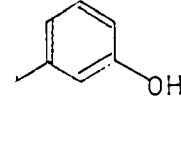
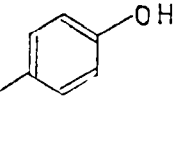


given in Fig. 52 were confirmed. The results are summarised in Table 11. A high retention of tritium is observed for all the products, except for p-cresol, which showed little migration and retention of tritium.

The oxidation of $[4-^2\text{H}]$ -anisole was studied under similar conditions. A mixture of 104 and an excess of

Table 11

Product distribution from the oxidation of $[4\text{-}^3\text{H, methyl-}^{14}\text{C}]\text{-toluene}$ ($^3\text{H}:^{14}\text{C}$ ratio = 17.5:1) with benz $[c,d]\text{-indazole 1,2-dioxide 104}$

Product						
Yield (% based on <u>104</u>)	40	19	0.5	11	3	± 4
$^3\text{H}:^{14}\text{C}$ ratio	16.2:1	16.5:1	16.2:1	16.3:1	16.2:1	± 3.5:1
% retention of ^3H	93	94	93	93	93	± 20

deuterated substrate was heated at 105°C in 1,1,2,2-tetrachloroethane. The results are summarised in Table 12.

Table 12

Product distribution from the oxidation of $[4-^2\text{H}]$ -anisole (88% ^2H) with benz $[c,d]$ -indazole 1,2-dioxide 104

Product	Phenol	2-hydroxy- -anisole	4-hydroxy- -anisole
Yield (% based on <u>104</u>)	14	13	4
% ^2H	90	83	4
% ^2H after $\text{Et}_3\text{N-H}_2\text{O}$ exchange	5.5	83	3

The yields and the degree of deuterium retentions in the phenolic products were examined by g.l.c.-mass spectrometry, by comparison with standards. 4-Hydroxy-anisole was virtually free from deuterium and this indicates an absence of NIH shift in this thermal aromatic hydroxylation ; when $[4-^2\text{H}]$ -anisole was irradiated in dichloromethane at 25°C with pyridine N-oxide ⁽¹⁷⁾ a 45% deuterium retention was observed. The relative products ratio was also different : Jerina and coworkers obtained a 1:2:1 relative ratio of 2-hydroxy-
-anisole:4-hydroxy-anisole:phenol. There is a predominance of p- over o-hydroxylation, whilst a 13:4 ratio of o-hydroxyl-

ated product against p-hydroxylated product is obtained with this thermal oxygen transfer reaction. Another interesting feature is the distribution of α - and β -naphthol obtained by heating 104 with naphthalene. The β -isomer prevails over the α one (2.6% over 0.6%) whilst the irradiation of pyridine N-oxide affords mixtures containing > 95% of α -naphthol (17).

8. CONCLUSIONS

A brief survey has been given on hydroxylation reactions which are thought to proceed via atomic oxygen. However, in certain cases, the chemical behaviour of the oxidising species appears to be different. The ground state triplet oxygen $O(^3P)$ generated by mercury sensitised decomposition of N_2O is a biradical species and its chemical behaviour in its reactions with olefins is in agreement with its predicted properties. The relatively poor amount of deuterium retention in the 4-hydroxy-anisole isolated (17) is evidence for its radical character in attacking aromatic rings.

The irradiations of pyridazine N-oxides afforded good yields of phenols (30-40%) (52,53). When double bonds and aromatic rings are present in the same molecule, the olefin epoxidation is favoured over the aromatic hydroxylation process and thus a certain degree of selectivity is observed. Unfortunately there are no data available on the

relative amounts of *o*-, *m*- and *p*-hydroxylation products with toluene ; the authors simply obtain a mixture of cresols . A mixture of α - and β - naphthols (1:0.8), in total yield of 20-30%, is obtained.

The irradiation of pyridine N-oxides with aromatic hydrocarbons produces arene oxides ⁽¹⁷⁾. Thus, 1,2-epoxy-naphthalene and a mixture of α - and β - naphthols ($\alpha > 95\%$ in a 1% total yield) is obtained from naphthalene. The deuterium retentions are high and almost identical to those of the microsomal aromatic hydroxylation (60%). The thermolysis of benz[c,d]-indazole 1,2-dioxide 104 ⁽⁶⁶⁾ converts [4-²H]-anisole into 2-hydroxy-anisole (13%) and 4-hydroxy-anisole (4%) with low deuterium retention and a 13:4 ratio of *o*- to *p*- product.

The NIH shift and the formation of arene oxides are the criteria which must be satisfied for any good chemical model of the naturally occurring aromatic hydroxylations involving cytochrome P 450 enzymes. The pyridine N-oxide photohydroxylation appears to satisfy both conditions and should therefore be a good mechanistic approach for determining the chemical nature of the oxidising species acting in Nature. The "oxene" species in the photolysis of aromatic N-oxides is postulated from the reaction products and intermediates, but to date it has never been seen, even by use of sophisticated spectroscopic techniques. On the other hand this method has certain disadvantages : the yield of hydroxylation products is poor, since the concomitant rearrangement reactions are prevalent ⁽³³⁾; complex

reaction mixtures are obtained and long purification techniques are necessary. The other chemical models give better yields but the NIH shift criterion is not satisfied or is satisfied only to a small extent.

D I S C U S S I O N

1. THE IRRADIATION OF PYRIDINE N-OXIDE AND OF ITS BORON TRIFLUORIDE ADDUCT

The major disadvantage observed in the photohydroxylation of aromatic substrates with pyridine N-oxide is the poor yield of hydroxylation. Streith and coworkers (48) claim a 15% yield of phenol from pyridine N-oxide, by irradiation in benzene. The extensive work done at the National Institutes of Health, Bethesda, Maryland, by Jerina and coworkers, does not appear to confirm such high conversions ; for example, irradiation of pyridine N-oxide and naphthalene (20:1) in dichloromethane afforded naphthalene 1,2-oxide (ca 1%) and a mixture of naphthols (ca 1%, 5:95 , β : α) (17).

In the gas phase irradiation of 2-methylpyridine N-oxide, the choice of wavelength influences the reaction (47) - a result that appears to be unique. Different wavelengths ($\lambda = 300-320$ nm, $\lambda = 254$ nm) do not appear to influence the course of the liquid phase photolysis of pyridine N-oxides (33): although the deoxygenation and the rearrangement reactions are known to proceed via two different electronic states (34,35) both wavelengths can be used indifferently. Nagakawa and coworkers (55) found a wavelength dependence in irradiating N^a, N^b -dimethyltryptamine and pyridine N-oxide ($\lambda = 254$ nm or visible light) : no deoxygenation took place with visible light.

The main obstacle against the efficient use of pyridine N-oxides in aromatic hydroxylations is caused by the rearrangement reactions, which account for most of the products.

The other unavoidable problem is the formation of tars. The irradiation of pyridine N-oxide is not a clean reaction ; besides giving many identifiable products, it always affords unidentifiable tars, probably resulting from secondary photochemical reactions. We hoped to improve the hydroxylation yields by photolysing derivatives of pyridine N-oxide which might give intramolecular hydroxylation such as 2-phenethylpyridine N-oxide 105 and 2-benzylpyridine N-oxide 106 (Fig. 55).

To gain experience with these reactions some of the experiments reported by Streith and coworkers were repeated. Pyridine N-oxide was irradiated in benzene at 320 nm. Typically, reactions were monitored by thin layer chromatography (t.l.c.). After a short period a complex mixture of reaction products was obtained. The reaction mixture was separated by preparative t.l.c. in order to isolate phenol. Its presence was indicated by the use of a reference sample and by i.r. analogies. The i.r. spectrum of purified (Analar) phenol contains the following bands : 3610, 3050, 1595, 1510, 1475, 1345, 1265, 1180, 1155, 1075, 1030, 1005, 890 and 640 cm^{-1} . The spectrum of the reaction product showed all these bands : the relative intensities and the band shapes were identical. The band present in the spectrum of pure phenol at 1345 cm^{-1} was obscured in the spectrum of the reaction product by an intense impurity band. The estimated yield of phenol from the reaction mixture was 0.8-2.4 mg, corresponding to a maximum experimental yield of 1%. The isolation and estimation of phenol

proved troublesome. There are two factors which must be considered when trying to isolate phenol from a reaction mixture for quantitative purposes. The first one is the volatility of phenol ; all workup and purification procedures involving evaporations either at atmospheric or reduced pressures should therefore be carried out with great care and procedures involving very low pressures (≈ 1 mmHg) should be avoided. In order to estimate the efficiency of the isolation procedure some control reactions were carried out. A known amount of purified phenol was dissolved in benzene and concentrated in a spinning band column (20 mmHg, 25°C). When the volume of the solution was reduced down to 5 ml the phenol was converted into its 2,5,6-tribromo derivative : almost a quantitative yield of the derivative was obtained (95%). When the phenol solutions were subjected to relatively low pressures (0.5 mmHg), it readily evaporated with consequent loss of material. The second source of discrepancy in estimating phenol is due to the large response of phenols (large size spots) in t.l.c. chromatography. Even tiny amounts of phenol run as large round spots and readily absorb iodine ; therefore a simple, quantitative evaluation, based on t.l.c., is extremely deceptive and must be discarded.

In order to recheck the yields of phenol obtained in the photohydroxylation of benzene with pyridine N-oxide a quantitative u.v. determination was carried out. Four solutions of purified (Analar) phenol of known concentrations in methanol were prepared. The u.v. spectrum of

phenol in alcohol gives an absorption band at 274 nm, with an $\epsilon_{\text{max}} = 1450$ (67). The spectra obtained are shown in Fig. 53 .

Fig. 53

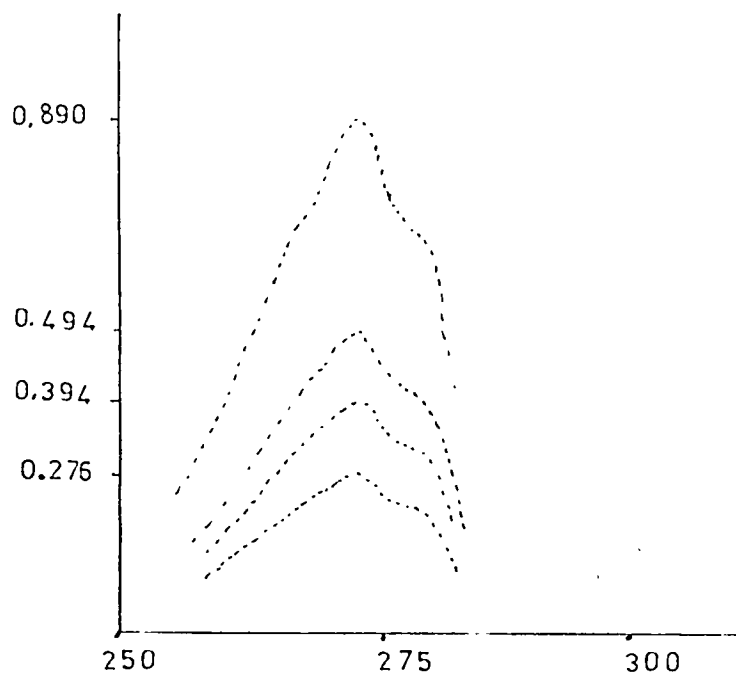


Table 13

Concentration of phenol in methanol solutions (x)	Absorbance values (y)
1) 1.56×10^{-2} g/l	0.276
2) 2.28×10^{-2} g/l	0.394
3) 2.88×10^{-2} g/l	0.494
4) 4.84×10^{-2} g/l	0.890

These experimental data (Table 13) represent a reasonable linear correlation. The best straight line through the points was calculated by the least squares method to

satisfy the equation $y = 13.9x - 0.03$

Pyridine N-oxide was irradiated at 320 nm in benzene and the yield of phenol calculated by u.v. spectroscopy from the control graph. The solution was concentrated at room temperature and phenol could again be detected by t.l.c.. The reaction mixture was extracted with aqueous base ; no phenol could be detected in the organic phase. The aqueous phase was acidified and extracted with ether ; these extractions are quite critical but controls with reference phenol did not reveal significant losses. After preparative t.l.c. a methanolic solution of phenol was obtained, which was used for the quantitative determination. Aliquots of the methanolic solution were estimated by u.v.. The average absorption value was 0.595, corresponding to a yield of 3.7 mg of phenol, viz 0.8 %.

In order to try to improve the yields an experiment using a Reading photolysis apparatus was attempted. The internal walls of such a reactor are continuously swept by an internal spinning cleaner ; the reaction tars cannot therefore stick on the walls of the reactor and so partially prevent the light from reaching the reacting solution. The reaction yields were checked by forming 2,4,6-tribromophenol, as normally employed for the quantitative analysis of phenols ⁽⁶⁸⁾. Some 2,4,6-tribromophenol was synthesised from phenol, with a similar workup procedure and the yield in pure 2,4,6-tribromophenol was almost quantitative (96%).

Pyridine N-oxide was photolysed in a 1:1 mixture of dichloromethane and benzene. A solvent mixture was used since the results of this experiment were to be compared

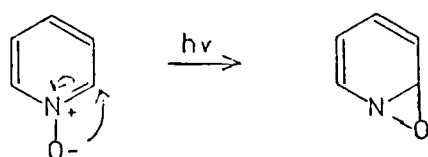
with the results of a later experiment, in which the boron trifluoride adduct of pyridine N-oxide was irradiated : this adduct is virtually insoluble in pure benzene, hence forcing the use of solvent mixtures. The spinning band column was used to concentrate the solution in order to avoid phenol losses. The t.l.c. analysis confirmed the formation of a product having the same R_f as phenol, as well as the same behaviour towards a u.v. lamp and towards iodine. After alkaline extraction and acidification of the aqueous phase, bromine was added and a precipitate immediately formed. After workup the reaction mixture was examined by t.l.c. and the presence of a compound having the same R_f as reference 2,4,6-tribromophenol was confirmed, together with the absence of reference phenol. The compound was purified by preparative t.l.c. and its structure was confirmed by its m.p. and i.r. spectrum. 2,4,6-Tribromophenol was obtained in our experiment in a yield of 1%. These results are summarised in Table 14 .

Table 14

Pyridine N-oxide (ml)	Solvent	N-oxide concentration	Phenol yield	Method of analysis
2.49	C_6H_6 (40 ml)	$6.2 \times 10^{-2} M$	0.8-2.4 mg (1%)	i.r.
4.94	C_6H_6 (50 ml)	$9.8 \times 10^{-2} M$	3.7 mg (0.8 %)	u.v.
12.62	$C_6H_6:CH_2Cl_2$ 1:1 (75ml each)	$8.4 \times 10^{-2} M$	45 mg (1%)	formation of 2,4,6-tribromophenol

The formation of the rearrangement products in the photolysis of aromatic N-oxides is likely to proceed via an oxaziridine intermediate (33). A lone pair from the oxygen atom (formally negatively charged) attacks a carbon atom α to the nitrogen of the pyridine ring (Fig. 54).

Fig. 54



The rearrangement products usually prevail over the deoxygenation products (33). For this reason the photolytic hydroxylation by aromatic N-oxides, although providing a good chemical model for biological hydroxylation proceeding with the NIH shift (16,17), cannot be used at all for synthetic purposes.

We hoped to improve the hydroxylation yields by favouring an intramolecular oxygen transfer process. As will be shown later, however, 2-phenethylpyridine N-oxide 105 and 2-benzylpyridine N-oxide 106 did not show much improvement with respect to pyridine N-oxide in benzene and the order of magnitude of the hydroxylation products was not different.

We hoped to block the oxaziridine formation (hence the rearrangement products) and to improve the deoxygenation yields by forming a complex between the N-oxide group and a Lewis acid. Some solid 1:1 complexes of pyridine N-oxide

with BX_3 , where $X = H$ or halogen, have been reported and examined by Kulevsky and Sveum⁽⁶⁹⁾, especially by i.r. spectroscopy. The photochemical behaviour of the boron trifluoride complexes of pyridine N-oxide, 2-phenethylpyridine N-oxide 105, 2-benzylpyridine N-oxide 106 and 2-phenoxy-pyridine N-oxide 124 has now been studied. The procedure adopted by Kulevsky and Sveum for forming such adducts consisted in passing gaseous boron trifluoride through a solution of pyridine N-oxide in chloroform. The boron trifluoride adducts can also be obtained by adding a stoichiometric amount of boron trifluoride-diethyl ether complex to a solution of the aromatic N-oxide in chloroform, the nuisance of using boron trifluoride gas being thus eliminated and quantitative yields being obtained in short times. The aromatic N-oxide-boron trifluoride adducts sometimes precipitate out of solution (pyridine N-oxide, 2-phenethylpyridine N-oxide) and the adducts of other N-oxides solidify after evaporation under reduced pressure (2-benzylpyridine N-oxide, 2-phenoxy-pyridine N-oxide, 2-butylpyridine N-oxide). A shift towards lower wavenumbers in the i.r. spectra of such adducts can be observed for the N-O stretching bands (Table 15). The $\Delta\nu_{NO}$ is larger in substituted pyridine N-oxides ; this factor could also facilitate the deoxygenation reaction, as the bond energy of the nitrogen-oxygen bond appears to be considerably weakened by the formation of the adduct with boron trifluoride - a $\Delta\nu_{NO}$ of 100, 115 and 80 cm^{-1} is observed in the boron trifluoride adducts of compounds 105, 106 and 124 (Table 15).

Table 15

Molecule	$\nu_{\text{NO}}(\text{cm}^{-1})$	$\Delta\nu_{\text{NO}}(\text{cm}^{-1})$	Solvent
Pyridine N-oxide BF ₃ adduct	1242 1217	25	nujol
4-Methylpyridine N-oxide BF ₃ adduct	1238 1127	111	nujol
2-Benzylpyridine N-oxide BF ₃ adduct	1265 1150	115	nujol
2-Phenoxypyridine N-oxide BF ₃ adduct	1240 1160	80	CHCl ₃
2-Butylpyridine N-oxide BF ₃ adduct	1250 1160	90	CHCl ₃
2-Phenethylpyridine N-oxide BF ₃ adduct	1250 1150	100	CHCl ₃

The n.m.r. spectra of the compounds 105, 106 and 124 differ from the n.m.r. of their corresponding boron trifluoride adducts. 2-Phenethylpyridine N-oxide 105 shows a multiplet at δ 8-8.2 and such a signal is attributed to the proton α to the nitrogen. The signal is shifted towards a low field in its boron trifluoride adduct (δ 8.5-8.65). An analogous phenomenon is observed in the n.m.r. spectrum of 2-benzylpyridine N-oxide 106 and of its adduct (the proton α to the nitrogen is shifted from δ 7.9-8.1 to δ 8.5-

-8.7). 2-Phenoxypyridine N-oxide 124 also shows such a shift : the signal at δ 8.15-8.22 is shifted to δ 8.5-8.7 in its boron trifluoride adduct.

The u.v. spectra of 2-phenethylpyridine N-oxide 105, of 2-benzylpyridine N-oxide 106 and 2-phenoxypyridine N-oxide 124 have been registered in chloroform (Table 16).

Table 16

Compound	$\lambda_{\max}$ (nm)	ϵ
<u>105</u>	273	14930
BF ₃ adduct of <u>105</u>	273	7540
<u>106</u>	272	14000
BF ₃ adduct of <u>106</u>	272	6600
<u>124</u>	313	2680
	271	7570
BF ₃ adduct of <u>124</u>	313	4517
	271	15056

The u.v. spectrum of 2-benzylpyridine N-oxide 106 was also run in aqueous dilute sulphuric acid. The protonated form showed an absorption at $\lambda_{\max}$ 263 nm, whilst an aqueous solution of 106 gave an absorption at $\lambda_{\max}$ 256 nm.

The boron trifluoride adducts of aromatic N-oxides can be destroyed either by treatment with water (an alkaline buffer) and extraction with an organic solvent, or by

stirring with alumina in an organic solvent, and filtering. The free N-oxides can be recovered quantitatively after evaporation of the solvent.

Pyridine N-oxide boron trifluoride adduct was irradiated in a benzene-dichloromethane mixture. The boron trifluoride adduct is highly insoluble in benzene, so necessitating the use of a solvent mixture. The reaction mixture was concentrated at the spinning band column ; after removing the boron trifluoride by stirring with alumina, a t.l.c. plate confirmed the formation of phenol, together with the absence of most of the rearrangement products present in the irradiation of pyridine N-oxide in benzene.

The 2,4,6-tribromo derivative of phenol was formed. The workup afforded some crystals (145 mg, 6.8%) and the m.p., i.r. and ^1H n.m.r. spectra were identical with a reference sample of 2,4,6-tribromophenol.

Pyridine N-oxide boron trifluoride was also irradiated in anisole. A dark violet colour immediately developed as soon as the solvent was added to the adduct, which was readily soluble in anisole (contrast with benzene and with toluene). A charge transfer complex between the electron poor aromatic ring of the adduct and the electron rich aromatic ring of anisole was thought to be responsible for the colour ; trials to detect the charge transfer complex by u.v. spectroscopy failed. After irradiation and workup the reaction mixture was separated into two components : a yellow oil (55 mg), which had the same spectral (i.r. and n.m.r.) and chromatographic (t.l.c.) properties of 2-hydroxy-anisole (6.9% yield), and another oil (3.5 mg) which appeared

to be a mixture of phenol (ca 0.5%)^{*} and another unidentified product by i.r. spectral assignments. The phenol must arise from demethylation of anisole. These results with the boron trifluoride complex, although not spectacular, were encouraging. An important feature is the distribution of products. The irradiation of pyridine N-oxide in benzene afforded very complicated mixtures. In this case the rearrangement products were not isolated and purified, since the aim of this experiment was only to check the reproducibility of the previous literature work (48).

An improvement in the yield of phenol (6.9% yield) has been obtained by irradiating the pyridine N-oxide BF_3 adduct. Furthermore, the latter reactions afforded fewer products and the purification problem was much simpler. A more careful examination of products has been carried out with 2-phenethylpyridine N-oxide 105 and with 2-benzylpyridine N-oxide 106. The chemistry of these compounds and of their boron trifluoride adducts under irradiation will be described in the next chapter. In these examples the formation of the rearrangement products was blocked by using their boron trifluoride adducts and the yields of the hydroxylation products improved considerably.

2. THE IRRADIATION OF 2-PHENETHYLPYRIDINE N-OXIDE, OF 2-BENZYL-PYRIDINE N-OXIDE AND OF THEIR BORON TRIFLUORIDE ADDUCTS

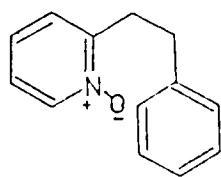
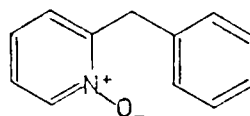
Enzymes are better operators than bench chemists: they work under extremely mild conditions, and they carry out reactions quickly and efficiently; furthermore their

*Total yields

reactions are regio and stereospecific. It is almost impossible to reproduce all these properties on a bench, but they stimulate and challenge the chemist.

Jerina and coworkers have found that the irradiation of pyridine N-oxide in naphthalene mimics certain essential features of the in vivo hydroxylation of naphthalene. The deuterium retention is extremely similar and an arene oxide intermediate is formed (16,17). These strong analogies in the reaction mechanism suggest an identity of the actual chemical species performing such a reaction. The enzymes produce it from molecular oxygen under very mild conditions. As scientists, we can obtain such oxidation by using pyridine N-oxide with u.v. light (that is not so mild!) and in poor yields. In the enzyme the substrate is placed at the right place at the right moment. Thus, when a very reactive species is generated, it cannot but react with the substrate at particular sites. The basic method is very simple and logical, yet difficult and cumbersome for imitation by bench chemists using chemical models. We hoped to improve the hydroxylation yields by trying to favour an intramolecular oxygen transfer mechanism. 2-Phenethylpyridine N-oxide 105 and 2-benzylpyridine N-oxide 106 were chosen as chemical models for this purpose (Fig. 55).

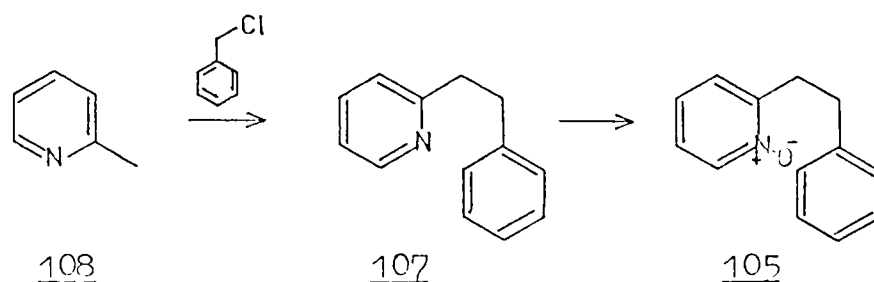
Fig. 55

105106

In model 105 the two aromatic rings may nearly overlap (as seen from Dreiding molecular models) and there is a good spatial interaction between the oxide function and the ortho positions of the phenyl ring. A good interaction between the same functions is also present in model 106. For this reason intramolecular hydroxylation reactions might be expected with both models 105 and 106.

2-Phenethylpyridine N-oxide 105 was prepared by the reaction sequence shown in Fig. 56.

Fig. 56

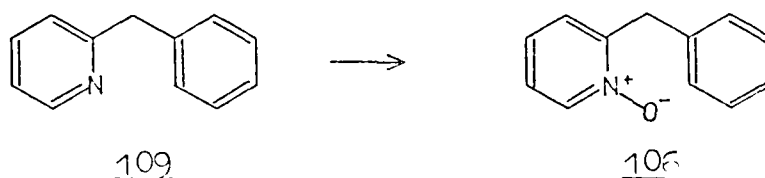


2-Phenethylpyridine 107 was prepared from 2-methylpyridine 103 and benzyl chloride, according to the procedure of Bergstrom, Norton and Seibert (70). Sodamide was used instead of potassium amide and a 62% yield of 2-phenethylpyridine 107 was obtained. The chromatographically pure (t.l.c.) compound was checked by n.m.r. ; the four benzylic protons gave a sharp signal at δ 3.1, and the proton α to the nitrogen resonated as a broad signal between δ 8.5 and 8.7. Part of the 2-phenethylpyridine 107 was transformed into 2-phenethylpyridine N-oxide 105 using the method of Ochiai (71). Compound 105 was obtained as colourless needles with m.p. $80.5\text{--}81^\circ\text{C}$. The i.r. data showed a broad, intense

band at 1255 cm^{-1} , due to the N-O stretching ; this band was absent in the i.r. spectrum of 2-phenethylpyridine 107. In the n.m.r. spectrum the proton α to the nitrogen was moved to higher field, from δ 8.5-8.7 in the free base to δ 8-8.2 in the N-oxide.

2-Benzylpyridine N-oxide 106 was obtained from 2-benzylpyridine 109 by the method of Katritzky and Hands⁽⁷²⁾ which involves a slight modification in the workup procedure of Ochiai⁽⁷¹⁾ (Fig. 57).

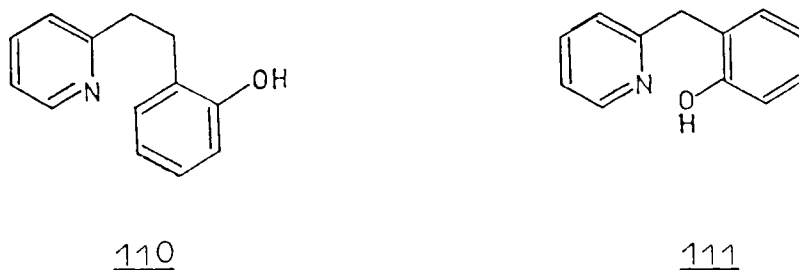
Fig. 57



Compound 106 was obtained as colourless needles (m.p. 100°C , literature m.p. 100.5°C) from a commercial sample of 2-benzylpyridine 109. The i.r. spectrum confirmed the N-oxidation : a strong and intense band at 1270 cm^{-1} , attributable to the N-O stretching band, appeared ; this band was absent in the i.r. spectrum of compound 109. The n.m.r. spectrum was also characteristic. The sharp signal due to the two benzylic protons was shifted toward lower field (from δ 4.02 in the free base 109 to δ 4.22 in the N-oxide 106) ; the multiplet attributable to the proton α to the nitrogen was shifted towards higher field (δ 8.44-8.55 in the free base 109, δ 7.9-8.1 in the N-oxide 106).

The irradiation of 2-phenethylpyridine N-oxide 105 and of 2-benzylpyridine N-oxide 106 did not produce high yields of the desired 2-(2'-o-hydroxyphenyl)ethylpyridine 110 and 2-(2'-hydroxyphenyl)methylpyridine 111. The main reaction products from the irradiation of 105 and 106 were isolated and their structures tentatively assigned. Some deoxygenated products 107 and 109 were isolated. All the other isolated products did not appear to contain phenolic hydroxyls. In order to detect whether at least traces of 110 and 111 (Fig. 58) were formed, authentic samples of these compounds were synthesised via independent routes.

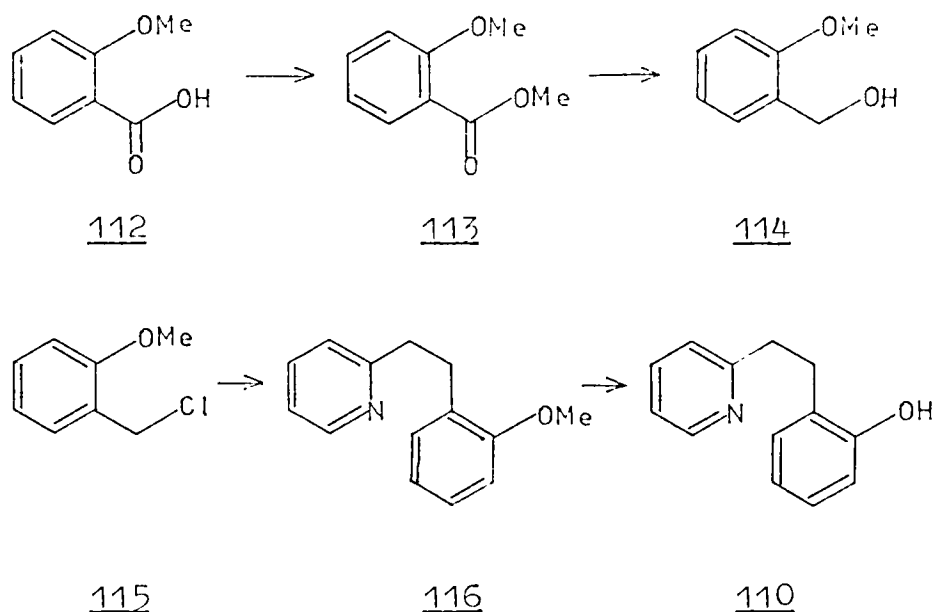
Fig. 58



On isolating and purifying the compounds having the same R_f as 110 and 111, from the complex photochemical reaction mixtures from 105 and 106, it was possible to prove the presence of these o-phenols, produced in very low amounts.

2-(2'-o-Hydroxyphenyl)ethylpyridine 110 was obtained through the reaction sequence shown in Fig. 59. o-Methoxybenzoic acid 112 was esterified to the ester 113 and the product was reduced to o-methoxybenzyl alcohol 114 with

Fig. 59

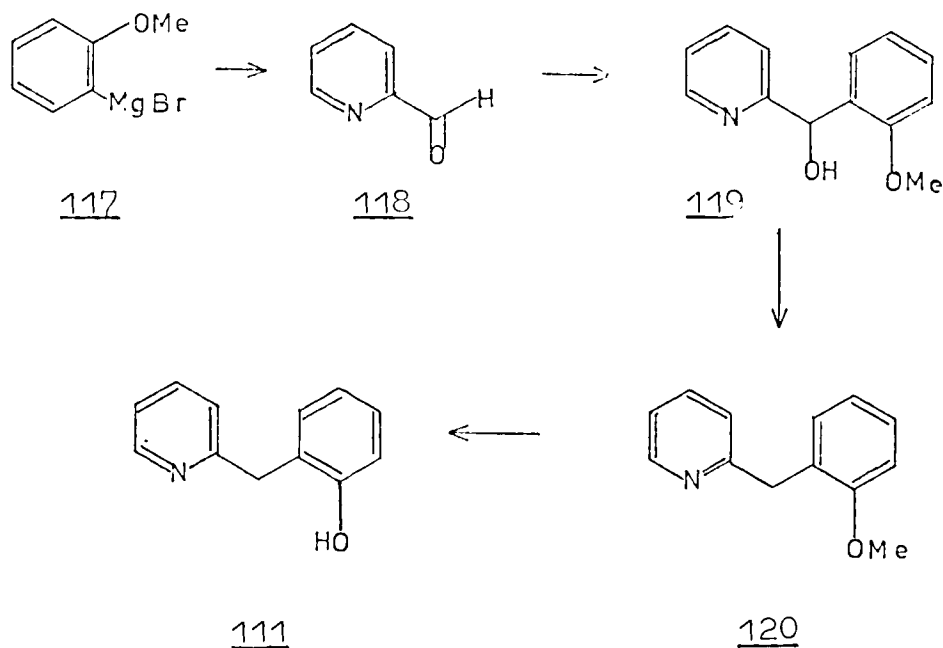


lithium aluminium hydride. The alcohol 114 was converted into o-methoxybenzyl chloride 115 with thionyl chloride. Finally 2-methylpyridine was condensed with the benzyl chloride 115. The methyl ether 116 was demethylated with boron tribromide, using the procedure of McOmie and Watts⁽⁷³⁾, and 2-(2'-o-hydroxyphenyl)ethylpyridine 110 was obtained. The structure of compound 110 was characterised by micro-analytical and spectral data. The i.r. and ^1H n.m.r. spectra suggest a strongly chelated hydroxyl group : a broad and complex system of bands appears in the i.r. spectrum, between 3500 and 2300 cm^{-1} , with maxima at 3090, 2940, 2850, 2780, 2715 and 2625 cm^{-1} . Solutions of 110 at various concentrations did not change their pattern of the band system, suggesting an intramolecular hydrogen bonding between the hydroxyl and

the nitrogen atom ⁽⁷⁴⁾. The ¹H n.m.r. spectrum also confirmed such a chelation. The phenolic proton resonates with a broad signal at a very low field (δ 9.66-9.16) : strongly chelated phenolic protons also resonate in a low field region ⁽⁷⁵⁾ (usual range of phenolic protons : δ 6.5-4.5). It is interesting to note the effect of the methoxy and of the hydroxy group on the ¹H n.m.r. signals on the benzylic protons in compounds 116 and 110. In the methyl ether 116 the four benzylic protons resonate with a sharp signal at δ 3.81. The demethylation of 116 gave 2-(2'-o-hydroxyphenyl)ethylpyridine 110 : the signals attributed to the benzyl protons show a splitting (separated by 2 Hz) and the base of such a system is considerably broadened and complicated. Such a splitting could be attributed to the chelation of the hydroxyl with the nitrogen atom ; an eight membered ring should be formed, with a relatively rigid conformation, due to the chelation and the two aromatic rings.

2-(2'-Hydroxybenzyl)pyridine 111 was obtained via the scheme shown in Fig. 60. The Grignard reagent 117 of o-methoxybromobenzene was condensed with 2-formylpyridine 118. The benzyl alcohol 119 that formed could not be cleanly reduced by hydrogenation; it was therefore converted into its tosylate and reduced in situ with lithium aluminium hydride. After purification 2-(2'-methoxyphenyl)methylpyridine 120 was obtained in low yields (17%). The methyl ether 120 was demethylated with boron tribromide and 2-(2'-hydroxyphenyl)methylpyridine 111 was obtained. The structure of 111 was confirmed by microanalytical and spectral data. The i.r. and ¹H n.m.r. spectra suggested strong intramolecular

Fig. 60



chelation, as in compound 110. The i.r. spectrum showed a broad system of bands, extending from 3500 to 2500 cm^{-1} , with maxima at 3150, 3030, 3040, 2970, 2870, 2590 and 2540 cm^{-1} ; the pattern did not appear to change at various concentrations. The ^1H n.m.r. spectrum showed the phenolic hydroxyl to resonate at a low field (δ 11.4-11.6, broad); this value is again typical of chelated phenolic hydrogens⁽⁷⁵⁾. It is interesting to compare the mass spectra of compounds 110 and 111. The two molecular ion peaks are present, together with the molecular ion minus one peaks. The acidic hydrogen is readily lost in both compounds 110 and 111. The most intense peaks are due to benzylic break in compound 110 (M/e 93, 100% ; M/e 106, 24%) and to loss of OH in

compound 111 (M/e 163, 100%). The peak due to loss of OH is also present in the mass spectrum of compound 110, although at a much lower intensity than the molecular ion itself (M/e 199, M^+ , 63%; M/e 187, $M^+ - OH$, 26%).

2-Phenethylpyridine N-oxide 105 was irradiated under a variety of conditions. The crude mixtures from these reactions always appeared complicated. A change of the wavelength used (320 or 257 nm) did not affect the type of product formation. In the initial experiments the isolation of components was carried out without an authentic sample of 2-(2'-o-hydroxyphenyl)ethylpyridine 110 as a reference. At this stage it was hoped that this desired product would be present in relatively large amounts. The purification technique employed was preparative t.l.c., and the main components were repeatedly purified (sometimes three or four successive purifications were necessary) until single compounds could be obtained. The isolated products were then examined spectroscopically. In one such experiment (320 nm) seven compounds were isolated. Some starting material (7.6%) was recovered, and deoxygenation also took place, 2-phenethylpyridine 107 being obtained in 18% yield. In other experiments where the wavelength and reaction times were changed, amounts of the deoxygenated material did not appear to vary substantially. The amount of deoxygenated product formed is in reasonable agreement with the time of irradiation (Table 17).

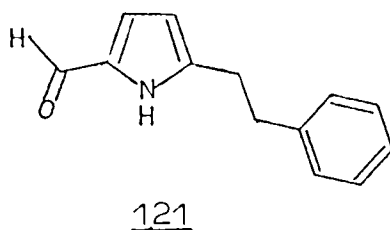
All the other five compounds gave mass spectra which showed strong peaks at M/e 91 and 103, suggesting that no

hydroxylation had occurred on the phenyl ring. Furthermore they all showed peaks at M/e 199, confirming them to be rearrangement products. All their i.r. spectra showed carbonyl absorptions. The structure of 2-formyl-5-phenethylpyrrole 121 was assigned to one of them (17%) (Fig. 61).

Table 17

Wavelength (nm)	Time (min.)	2-Phenethylpyridine
320	30	12%
320	120	18%
254	30	10%
254	120	23%

Fig. 61



The ^1H n.m.r. spectrum suggested structure 121, showing signals at δ 3.02 (4H, s, ethano bridge), 6.1 and 6.9 (2H, m, pyrrole protons), 9.4 (1H, s, aldehyde) and a broad signal at δ 12.0 (1H, NH, exchangeable with D_2O).

The i.r. spectrum showed, *inter alia*, bands at 3280 ($\nu_{\text{N-H}}$) and at 1660 cm^{-1} (carbonyl stretching) : these values also supported structure 121 (76). The mass spectrum showed peaks at M/e 199 (M^+ , 48%), 170 (loss of CHO , 16%), 108 (benzylic cleavage, 100%), 91 (benzylic cleavage, 17%) (77).

The total yield of all the rearrangement products is of the order of 40%. A similar amount of polar, tarry material also formed, and about 18% of the deoxygenated material. Thus the yield of the required aromatic hydroxylation product have to be low. Other experiments confirmed the reproducibility of such yields.

In order to discover whether at least traces of 2-(2'-*o*-hydroxyphenyl)ethylpyridine 110 formed, the mixture was purified with the help of a reference sample of authentic phenol 110, synthesised by an independent route (Fig. 59). In this way a small amount of *o*-hydroxylated product could be detected, isolated in extremely poor yields (0.6%), which were of the same order of magnitude as the yield of phenol obtained from the irradiation of pyridine N-oxide in benzene.

In order to check whether 2-phenethylpyridine N-oxide 105 was not producing at least modest yields of aromatic hydroxylation because of unfavourable conformational reasons, the reaction was repeated in benzene and examined for phenol and for compound 110. An amount of deoxygenated product 107 was obtained (13%), but no 2-(2'-*o*-hydroxyphenyl)ethylpyridine 110 could be isolated. Phenol was detected, as a mixture with another compound, but the amount of this was not accurately quantified ; an i.r. analysis conclusively

showed its presence, although yields were <1%.

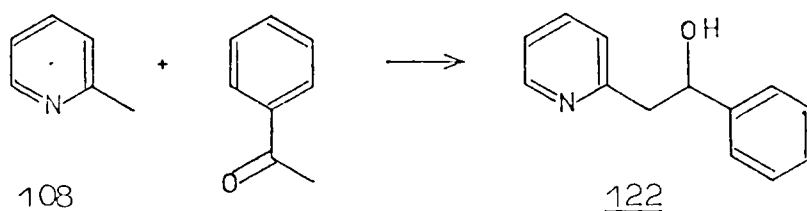
It was concluded that 2-phenethylpyridine N-oxide 105 was a bad model for an eventual intramolecular hydroxylation. Deoxygenation took place, but the aromatic ring was possibly in an unfavourable position for attack.

When 2-benzylpyridine N-oxide 106 was irradiated under similar conditions, 2-(2'-hydroxyphenyl)methylpyridine 111 could be isolated in slightly better yields (4%). 2-Benzylpyridine 109 was also formed (5%). The ratio of deoxygenated product 109 versus hydroxylated compound 111 showed an improvement with respect to the 2-phenethylpyridine N-oxide 105 model. The 2-(2'-hydroxyphenyl)methylpyridine 111 structure was proved by comparison with an authentic sample, prepared by an independent route (Fig. 60). The strong intramolecular chelation of the phenols 110 and 111, shown between the phenolic hydroxyl group and the nitrogen atom, was reflected in their t.l.c. behaviour : 2-(2'-o-hydroxyphenyl)ethylpyridine 110 is only slightly more polar than 2-phenethylpyridine 107, whilst the reverse occurs for 2-benzylpyridine 109 and 2-(2'-hydroxyphenyl)-methylpyridine 111.

The photochemistry of the boron trifluoride adducts of both 2-phenethylpyridine N-oxide 105 and 2-benzylpyridine N-oxide 106 were studied. The reactions were generally far cleaner and selective ; mainly deoxygenation and hydroxylation products were obtained, whilst the formation of the rearrangement products was virtually suppressed. The irradiation (254 nm) of the complex of 2-phenethylpyridine N-oxide 105

afforded 2-phenethylpyridine 107 (18.9%), 2-(2'-o-hydroxy-phenyl)ethylpyridine 110 (15.7%) and another isomeric compound 122 (15.4%). The total amount of deoxygenation products was 50%. Such yields are calculated from the reacted N-oxide. A considerable amount of starting material remained at optimum irradiation times and some tars also formed, which were probably the result of overreaction of products. However, a clean t.l.c. pattern was obtained and no rearrangement products could be isolated. The compound 122 was identified as an isomer to the phenol 110 from its mass spectral properties. The n.m.r. pattern indicated substitution of the ethano bridge, whilst its i.r. indicated the presence of an hydroxyl group. Thus two structures could be formulated for this compound. Assignment as the benzylic isomer 122 was confirmed by preparation of an authentic sample via the scheme shown in Fig. 62.

Fig. 62

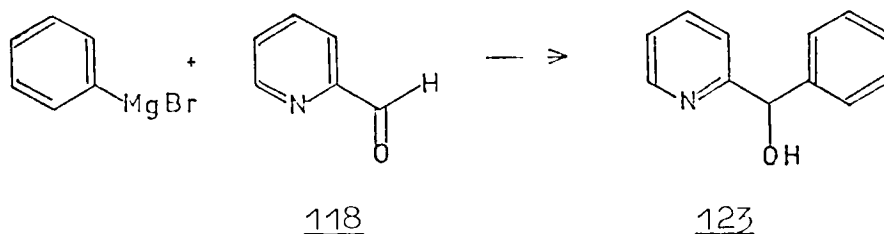


The mass spectrum showed the M/e 107 and 92 peaks, placing the hydroxyl group adjacent to the phenyl ring. The ¹H n.m.r. spectrum showed the benzylic proton as a triplet, centered at δ 5.2, and the other methylenic protons adjacent to the pyridine ring as a doublet, at δ 3.13 (J = 6 Hz). Decoupling

experiments confirmed this assignment. None of the isomeric benzyl alcohol or the *m*- and *p*-hydroxylated products (>1%) were detected in the reaction mixture.

The irradiation (254 nm) of the boron trifluoride complex of 2-benzylpyridine N-oxide 106 in dichloromethane gave three products identified as 2-(2'-hydroxyphenyl)methylpyridine 111 (43.6%), 2-benzylpyridine 109 (7.6%) and an alcohol 123 (4%). These yields are overall yields ; the starting material was not recovered and quantified. The alcohol 123 appeared from spectral evidence to be the benzylic species 123. Comparison with an authentic sample, prepared as indicated in Fig. 63, proved identity (R_f , m.p., and n.m.r. spectra).

Fig. 63



An overall deoxygenation yield of 55% was obtained. The absolute yields, based on recovered starting material, could not be calculated as the remaining starting material was not isolated from the reaction mixture. The absence of other rearrangement products was again noted in the irradiation of the boron trifluoride complex of 2-benzylpyridine N-oxide 106.

The inhibition of the rearrangement products derived from the oxaziridine appeared to be a general phenomenon under the influence of acids. An aqueous solution of sulphuric acid was also effective. Only small amounts of rearrangement products could be found during irradiation under these conditions, the products isolated being 2-(2'-hydroxyphenyl)methylpyridine 111 (15.9%), 2-benzylpyridine 109 (6.4%) and 2-(α -hydroxy, α -phenyl)methylpyridine 123 (3.8%), together with some starting material. Under such conditions, however, a greater amount of tar was formed.

An overall N-deoxygenation of 16% is obtained from the complex of 2-phenethylpyridine N-oxide 105, after a four hour irradiation period (254 nm), 64% of the starting N-oxide remaining unchanged. The ratio of hydroxylated products to 2-phenethylpyridine 107 is 1.62. In comparison an overall N-deoxygenation of 55% is obtained from the boron trifluoride complex of 2-benzylpyridine N-oxide 106. The ratio of hydroxylated products to 2-benzylpyridine is 11.

A great improvement was therefore obtained with 2-benzylpyridine N-oxide 106 : the yields of aromatic hydroxylation improved remarkably. The change from the free N-oxide to its boron trifluoride adduct gave an increase in o-hydroxylation of from 4 to 43.6%. The isolation and purification of the products was also greatly simplified, as the formation of the other rearrangement products was virtually suppressed. The formation of m- and p-hydroxylation products were at the most minimal, although searched for. The sensitivity of the purification techniques employed

did not permit the recognition and identification of such products.

The data so far collected suggested an intramolecular oxygenation mechanism, with the generation of a very reactive oxidising agent, showing electrophilic character. The reaction appeared sensitive to stereochemical factors. The length of the side chain has a considerable effect on the hydroxylation yields : 43% of 2-(2'-hydroxyphenyl)methylpyridine 111 was obtained against 15% of 2-(2'-o-hydroxyphenyl)ethylpyridine 110. Furthermore 15% of 2-(2'-hydroxy-2'-phenyl)ethylpyridine 122 was obtained against 4% of 2-(α -hydroxy- α -phenyl)methylpyridine 123. Such reactions therefore appear to be quite regiospecific and this could well be explained by the formation of atomic oxygen.

Various questions arose :

- a) Is the process intramolecular ?
- b) Is there formation of arene oxides ?
- c) What is the actual oxidising species ?

We tried to give an answer by examining the behaviour of the boron trifluoride complex of 2-phenoxy pyridine N-oxide 124.

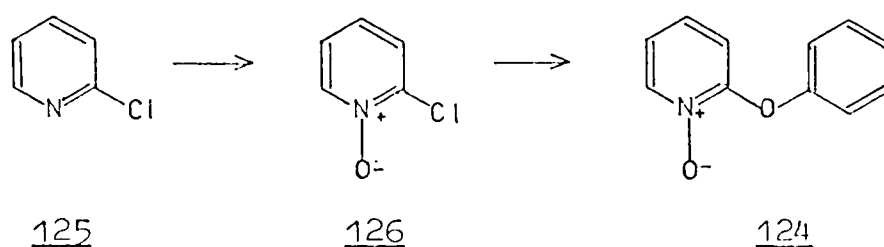
3. THE IRRADIATION OF THE BOMON TRIFLUORIDE COMPLEX OF 2-PHENOXYPYRIDINE N-OXIDE

We hoped to find a chemical model for the directed hydroxylation of aromatic substrates in which :

- 1) the aromatic substrate could easily be attached to the hydroxylating species
- 2) the hydroxylation would take place in high yields and possibly with a high degree of o-selectivity
- 3) the hydroxylated substrate should be released easily from the hydroxylating species.

2-Phenoxypyridine N-oxide 124 was thought to satisfy all these requirements, and was synthesised according to the sequence shown in Fig. 64.

Fig. 64



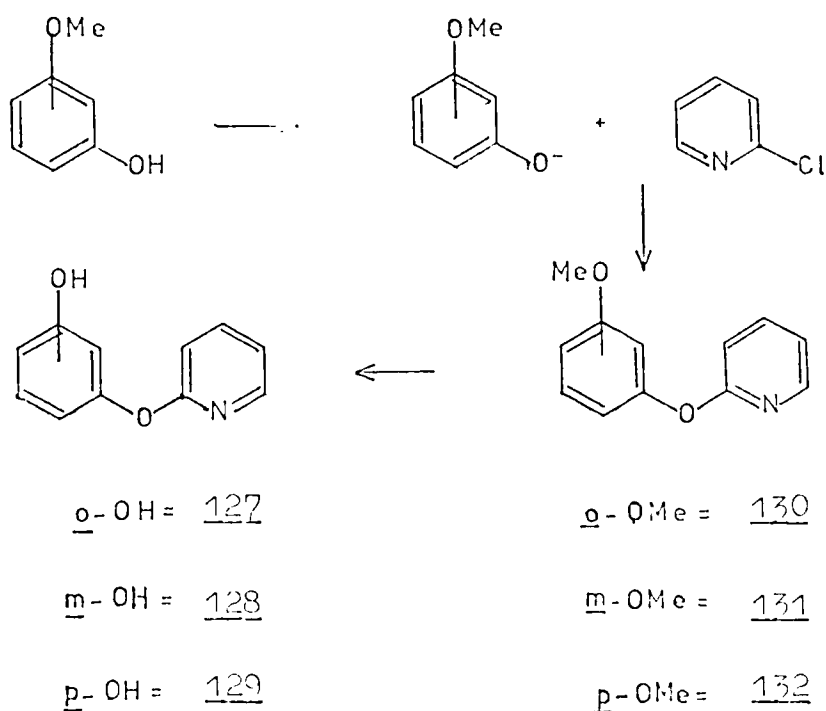
2-Chloropyridine N-oxide 126 was synthesised according to Katritzky's procedure ⁽⁷⁸⁾ in 70% yield. 2-Chloropyridine N-oxide 126 and sodium phenate were condensed in dimethylformamide under mild conditions (65°C, 2 hours). A 60% yield of purified 2-phenoxypyridine N-oxide 124 was

obtained, but these yields could be easily improved by using longer purification procedures. The ether 124 was characterised by spectral methods ; its mass spectrum showed peaks due to H^+ , to loss of oxygen from the molecular ion (M/e 171, 51.3%) and to the loss of hydroxyl group (M/e 170, 100%).

A reference sample of 2-phenoxypyridine was obtained by condensing 2-chloropyridine 125 with sodium phenate. A 61% yield of redistilled 2-phenoxypyridine was obtained as colourless crystals with m.p. 42°C .

The three o-, m- and p-phenols 127, 128 and 129 were similarly synthesised, according to the sequence shown in Fig. 65.

Fig. 65



Attempts to produce the compounds 127, 128 and 129 by direct monoarylation of the diphenols gave tarry mixtures, forcing the employment of the protected monomethylethers and subsequent selective demethylation ⁽⁷³⁾ of compounds 130, 131 and 132. The *o*-hydroxyether 127 for instance showed a singlet at δ 8.3, exchangeable with D₂O, which was assigned to the phenolic hydroxyl. Its low field position, compared to that in the *p*-hydroxyether 129 (δ 7.2), suggests a chelation to the nitrogen atom ⁽⁷⁵⁾. This chelation appeared in its i.r. spectrum, which shows a complex and broad system from 3590 to 2500 cm⁻¹ ⁽⁷⁴⁾. The chelation was also reflected by the t.l.c. behaviour of compounds 127, 128 and 129: the *o*-phenol 127 is less polar than the other isomers 128 and 129. It was not possible to separate the *m*-compound 128 from the *p*- one, 129, by t.l.c., although their separation was subsequently found to be possible by h.p.l.c. (Corasil II column).

The irradiation of the boron trifluoride adduct of 2-phenoxy-pyridine N-oxide 124 at 320 nm afforded 2-phenoxy-pyridine (20%), 2-(2'-hydroxyphenoxy)pyridine 127 (41%) and 2-(4'-hydroxyphenoxy)pyridine 129 (8.8%) (yields calculated for reacted material). The total yields were 17% (2-phenoxy-pyridine), 34% (127) and 7.2% (129). The purification technique employed in this first experiment was preparative t.l.c. and therefore the presence of any small amounts of 2-(3'-hydroxyphenoxy)pyridine 128 could not be estimated. The reaction products were identified by comparison with the authentic specimens.

An overall nitrogen deoxygenation of 70% (calculated

from the reacted N-oxide complex) took place. An o-/p-hydroxylation ratio of 4.7 was found ; the p-hydroxylated product was not found in the irradiation of 2-benzylpyridine N-oxide boron trifluoride. The formation of the m-hydroxylated product 123 was later indicated amongst the products by using h.p.l.c. technique : only a small percentage was formed ($\approx 1\%$).

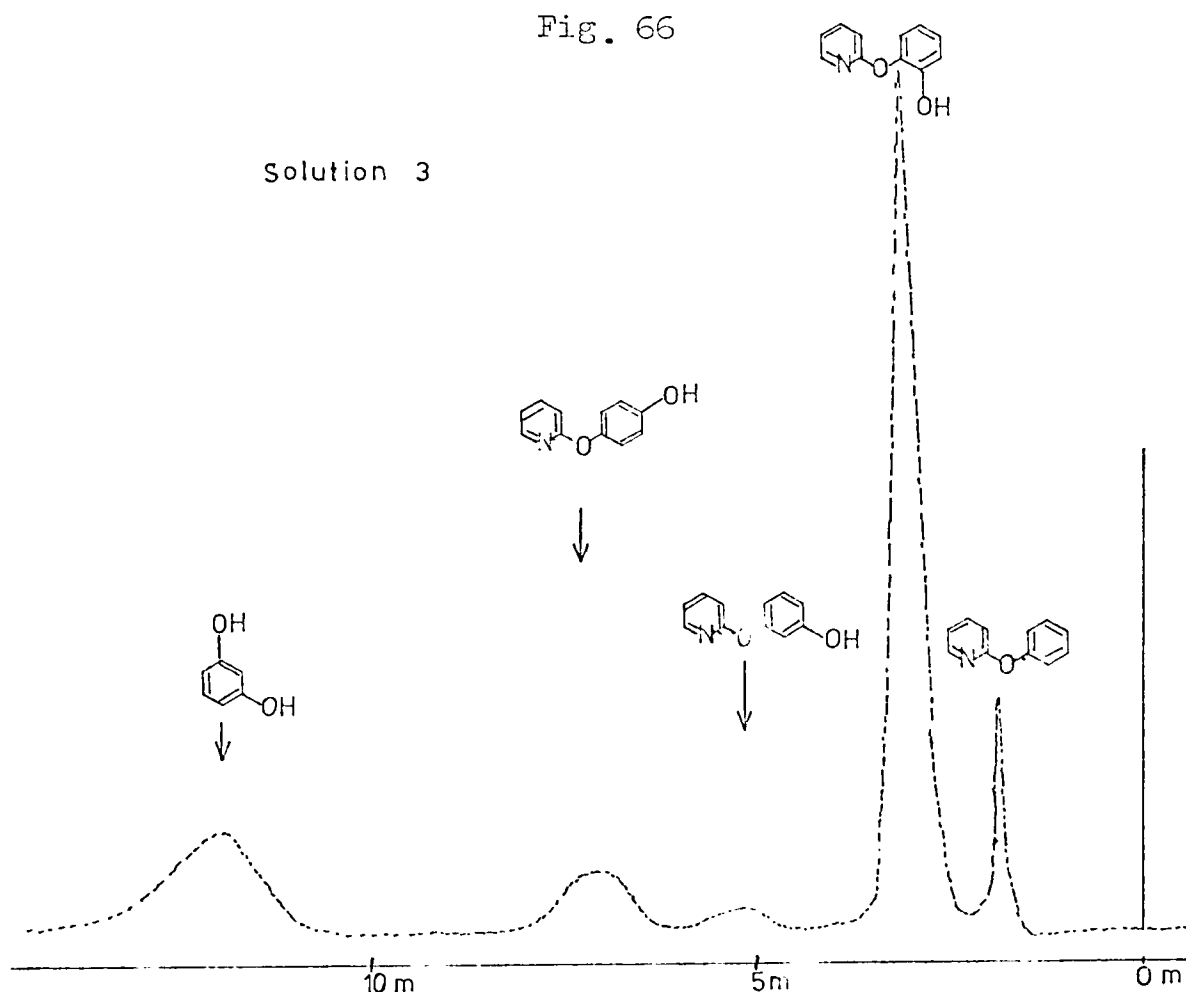
A quantitative estimation of 2-(2'-hydroxyphenoxy)pyridine 127 was determined by the h.p.l.c. technique, using 3-hydroxyphenol as an internal standard ; its retention time was longer than those of the reaction products. A reference graph was plotted from the u.v. absorptions of six solutions containing known amounts of 2-(2'-hydroxyphenoxy)pyridine 127 and 3-hydroxyphenol. The solutions were injected and a plot of the peak area ratios (on the x axis) versus the mole ratios (on the y axis) was obtained. A standard curve was obtained using the least squares method ($y = 0.180x$).

Five different concentrations of the boron trifluoride complex of 2-phenoxy pyridine N-oxide 124 were irradiated (320 nm) (Table 18).

Table 18

Solutions	Concentration	Irradiation time (h)
1	$0.21 \times 10^{-2} \text{M}$	10
2	$0.27 \times 10^{-2} \text{M}$	10
3	$0.73 \times 10^{-2} \text{M}$	10
4	$1.36 \times 10^{-2} \text{M}$	10
5	$2.07 \times 10^{-2} \text{M}$	18

After appropriate workup, a known amount of the 3-hydroxyphenol reference was added to each solution. The solutions were injected in the h.p.l.c. systems and five chromatograms were obtained (Fig. 66).



Peak areas were obtained by the weighing procedure. On using the standard curve previously obtained ($y = 0.180x$) the five experimental values for the yields of the o-hydroxylated product were 36%, 23%, 30%, 28% and 30% (these are total yields ; the starting material was not recovered and quantified). These results appear quite reproducible within experimental error ($\pm 15\%$). The first and largest amount was obtained from the least concentrated solution and can

be explained by the lower amount of starting material with respect to the other four solutions. The low amount (23.3%) of the required 2-(2'-hydroxyphenoxy)pyridine 127 obtained from the second solution involved a slightly different workup procedure. the crude reaction mixture was filtered through a silica column, instead of being stirred over alumina (in order to eliminate boron-trifluoride from the reaction mixtures).

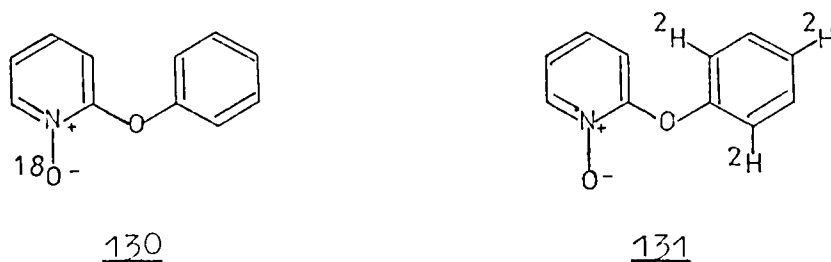
It is also of importance to compare the relative ratios of the o-/m- and o-/p- hydroxylation products. An intramolecular oxygen transfer mechanism should not in fact alter them. Unfortunately the amount of 2-(3'-hydroxyphenoxy)pyridine 128 produced was too small, and the error made by cutting the peaks, weighing them, and calculating the o-/m- peak area ratios was too large ($\pm 22\%$) so the o-/m- peak area ratios appear meaningless. The o-/p- ratios appear more reproducible and the percent errors are smaller ($\pm 9\%$). They were 5.0, 5.0, 4.7, 4.9 and 6.4, going from the most dilute to the most concentrated solution respectively. The value 6.4 is obtained from a solution irradiated for 18 h, whilst the four previous values are obtained from solutions irradiated for 10 h. From these results one cannot discount an intramolecular hydroxylation mechanism, although the experimental error is too large for any definite conclusions.

The boron trifluoride complex of 124 was irradiated in the presence of benzene : phenol was formed as traces (t.l.c.). Its identification by h.p.l.c. technique was not possible, as its retention time on our system was too

similar to the one of compound 127. The yield of 127, determined by h.p.l.c., was 27% ($\pm 13\%$).

In order to reach conclusive results for the involvement of either an inter or an intramolecular oxygen transfer mechanism, $[^{18}\text{O}]$ -N-labelled 2-phenoxy pyridine N-oxide 130 and 2- ($[2',4',6'-^2\text{H}_3]$ phenoxy) pyridine N-oxide 131 were synthesised (Fig. 67).

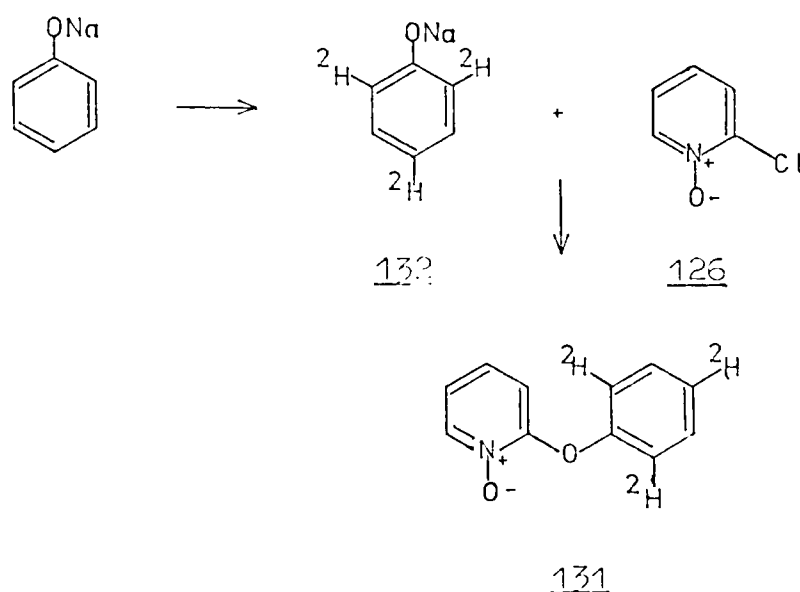
Fig. 67



An irradiation of a mixture of 130 and 131 should conclusively demonstrate an intramolecular hydroxylation mechanism : products having both ^{18}O and deuterium should not be formed. The irradiation of the complex of 131 would also provide light on the actual mechanism : if an arene oxide intermediate is formed, the resulting hydroxylated products (o- and p-) should retain more than two deuterium atoms, whilst the m-isomer should show partial deuterium loss. The deuterated N-oxide 131 was prepared according to the scheme shown in Fig. 68. $[2,4,6-^2\text{H}_3]$ phenol was prepared by heating sodium phenate in D_2O with a catalytic amount of sodium tert-butoxide. The exchange was shown by n.m.r. ; the complex multiplet shown by phenol between δ 6.7 and

and 7.35 is simplified by a broad singlet at δ 7.2 (2H). The labelled phenol was converted into its sodium salt 132 in dimethylformamide with sodium hydride, and condensed in situ with 2-chloropyridine N-oxide 126.

Fig. 68



A crude yield of 81% in 2-([2',4',6'- $^2\text{H}_3$] phenoxy)pyridine N-oxide 131 was obtained. The deuterium incorporation is confirmed by its mass spectrum; M^+ (M/e 190, 0.57%) and the peak due to simple loss of hydrogen (M/e 189, 1.95%) are present. An amount of dideuterated compound should give a peak at M/e 188 (see the mass spectrum of 2-phenoxy-pyridine N-oxide 124): such a peak is absent*. The loss of oxygen gives a peak at M/e 174 (60%). M/e 173 (51.7%), 172 (100%) and 171 (28.3%) were attributed to loss of OH, OD and HDO respectively

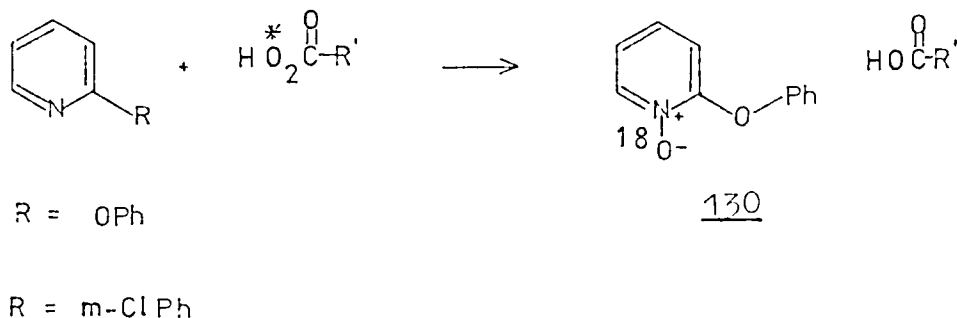
The boron trifluoride complex* of 131 was irradiated

* The intensity of such peaks is too low for quantitative determinations ; anyhow they give a qualitative idea .

in dichloromethane and the three isomeric phenols separated by preparative t.l.c. and by h.p.l.c.. The m- and the p-isomers could not be completely separated. The o-hydroxylation proceeded with the occurrence of the NIH shift. The high resolution mass spectral data showed the deuterium shift (M/e 190, molecular ion with three ^2H , and ^{13}C isotopic peak of the molecular ion of the dideuterated species, 24%). The M/e 189 peak was also present ; this peak is attributed to the molecular ion of the dideuterated species (34.65%). The peaks at M/e 173 (loss of OH from the $^2H_3$ species, and ^{13}C isotopic peak of M/e 172, 56.54%) and 172 (loss of OH from the $^2H_2$ species) are also useful for quantitative considerations. From these data about 40% of the initial deuterium content appears to have been retained in the o-hydroxylated product. The deuterium content of the m- and p-hydroxylated compounds was calculated by high resolution mass spectrometry at 10 eV. Such an ionisation potential does not fragment the molecule and only M^+ appears. The spectra of the unlabelled compounds 128 and 129 were run in analogous conditions. A comparison allowed to calculate the deuterium content in the labelled samples. Such a method for quantitative isotopic determinations is described by Biemann (88). The m-compound showed a $^2H_2$ abundance of 39% and a $^2H_3$ abundance of 39%. The p-compound showed a $^2H_2$ abundance of 38% and a $^2H_3$ abundance of 46%.

The $[^{18}\text{O}]$ -N-labelled N-oxide 130 was obtained through the scheme shown in Fig. 69.

Fig. 69

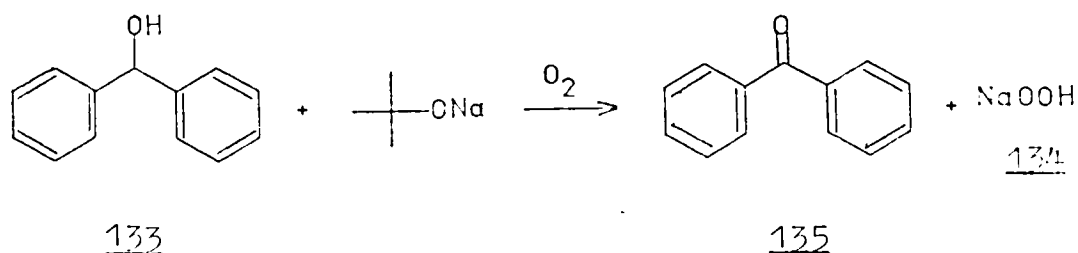


$[^{18}\text{O}]$ -N-Labelled aromatic N-oxides had not been, as far as known, synthesised in the literature. Our approach involved the synthesis of an $[^{18}\text{O}]$ -labelled peracid, which might be used for general ^{18}O labelling purposes as well. $[^{18}\text{O}]$ -Labelled peracids can be synthesised from $[^{18}\text{O}]$ -labelled hydrogen peroxide and the corresponding acid chloride or anhydride.

$[^{18}\text{O}]$ -Labelled hydrogen peroxide is not available on the market and the synthetic methods described in the literature do not give good yields. For instance solutions of $[^{18}\text{O}]$ -labelled H_2O_2 were prepared in up to 0.02M concentration by autooxidation of NH_2OH in the presence of ClO_4^- ions, followed by treatment with $^{18}\text{O}_2$ gas. The overall yield, based on $^{18}\text{O}_2$ used was 25-30% in six experiments under optimum conditions (79).

The autooxidation of alkaline alkoxides gives high yields of alkaline peroxidic compounds (80). Sodium hydroperoxide 134 is obtained in high yields (83%) from benzhydrol 133 (Fig. 70). Such a method allowed us to obtain aqueous solutions of sodium hydroperoxide (1.4M) with a 66% conversion of $^{16}\text{O}_2$ gas into sodium hydroperoxide.

Fig. 70

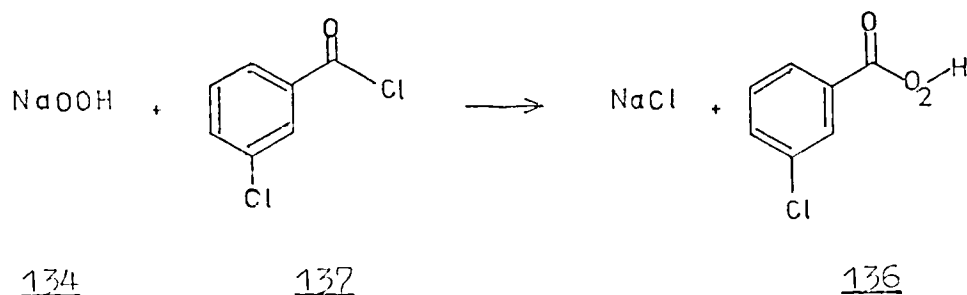


According to the literature (80), an equimolar amount of benzhydrol 133 and sodium t-butoxide absorbs slightly less than the stoichiometric amount of oxygen; benzophenone 135 is obtained as the oxidation product of benzhydrol, the absorbed oxygen being transformed into sodium hydroperoxide 134, insoluble in the reaction mixture. According to the reference (80), an 83% yield (calculated from benzhydrol) is obtained; such a yield was obtained by iodometric dosage. If $^{18}\text{O}_2$ gas is used, labelled sodium hydroperoxide can be obtained in high yields. We repeated the reaction several times with $^{16}\text{O}_2$ and a 70% average yield of sodium hydroperoxide 134 has been obtained; the yield was calculated through iodometric titration and unreacted benzhydrol was

still found at the end of the reaction (t.l.c.). Our reaction was best carried out using benzhydryl 133 and sodium t-butoxide in petroleum. A small amount of water containing a catalytic amount of magnesium sulfate (Mg^{2+} is known to stabilise peroxidic compounds ⁽⁸¹⁾) was added at the end of the reaction. The solid sodium hydroperoxide quickly dissolved on stirring and was easily separated as an aqueous solution.

Sodium hydroperoxide 134 was converted into *m*-chloro-perbenzoic acid 136 according to the procedure of Mc Donald, Steppel and Dorsey ⁽⁸²⁾ (Fig. 71). Their original hydro-

Fig. 71



peroxide:acid chloride ratio (3:1) was altered, in order to use as much labelled hydroperoxide as possible. Different sodium hydroperoxide:acyl chloride ratios were tried but unfortunately a large hydroperoxide excess is necessary, in order to have solutions with high N-oxidation power. As the reaction proceeds, the concentration of ArCO_2^- rises, while

that of HO_2^- drops, and a considerable amount of anhydride is formed ($\text{ArCO}_3^- + \text{ArCOCl} \longrightarrow \text{ArCO}_3\text{COAr} + \text{Cl}^-$). Unless an excess of HO_2^- is present, a mixture of peranhydride and peracid is obtained, and this accounts for the low N-oxidation yields obtained when a $\text{HO}_2^-/\text{RCOCl}$ ratio ≈ 1 has been tried. Solutions of labelled peracid having a good N-oxidation power ($\approx 70\%$) were obtained with a 1.8 ratio hydroperoxide/acid chloride.

The acid chloride 137 was dropped on the freshly prepared solution of sodium hydroperoxide 134, water-dioxan being used as the solvent. The solution was then acidified, extracted with dichloromethane and concentrated in vacuo at low temperatures. 2,6-Di-*t*-butyl-4-methylphenol was added to the organic phase before concentrating, in order to avoid radical decomposition of the peracid (83). In such a way about 54% of the hydroperoxide was converted into a mixture of *m*-chloroperbenzoic acid 136 and *m*-chloroperbenzoic anhydride (present in low amounts).

2-Benzylpyridine 109 was repeatedly oxidised in some control experiments using $^{16}\text{O}_2$. The dichloromethane solution of peracid was titrated iodometrically and an equivalent amount of 2-benzylpyridine 109 was added : a 69% conversion of 2-benzylpyridine N-oxide 106 was obtained (yield calculated by n.m.r.).

The experiment carried out with $^{18}\text{O}_2$ gas (21.3 ^{18}O atom percent) and 2-phenoxy-pyridine afforded a 50% yield of isolated, analytically pure [^{18}O]-N-labelled N-oxide 130 (obtained as a mixture with compound 124). The ^{18}O incorporation was calculated by mass spectral data ; high resolution

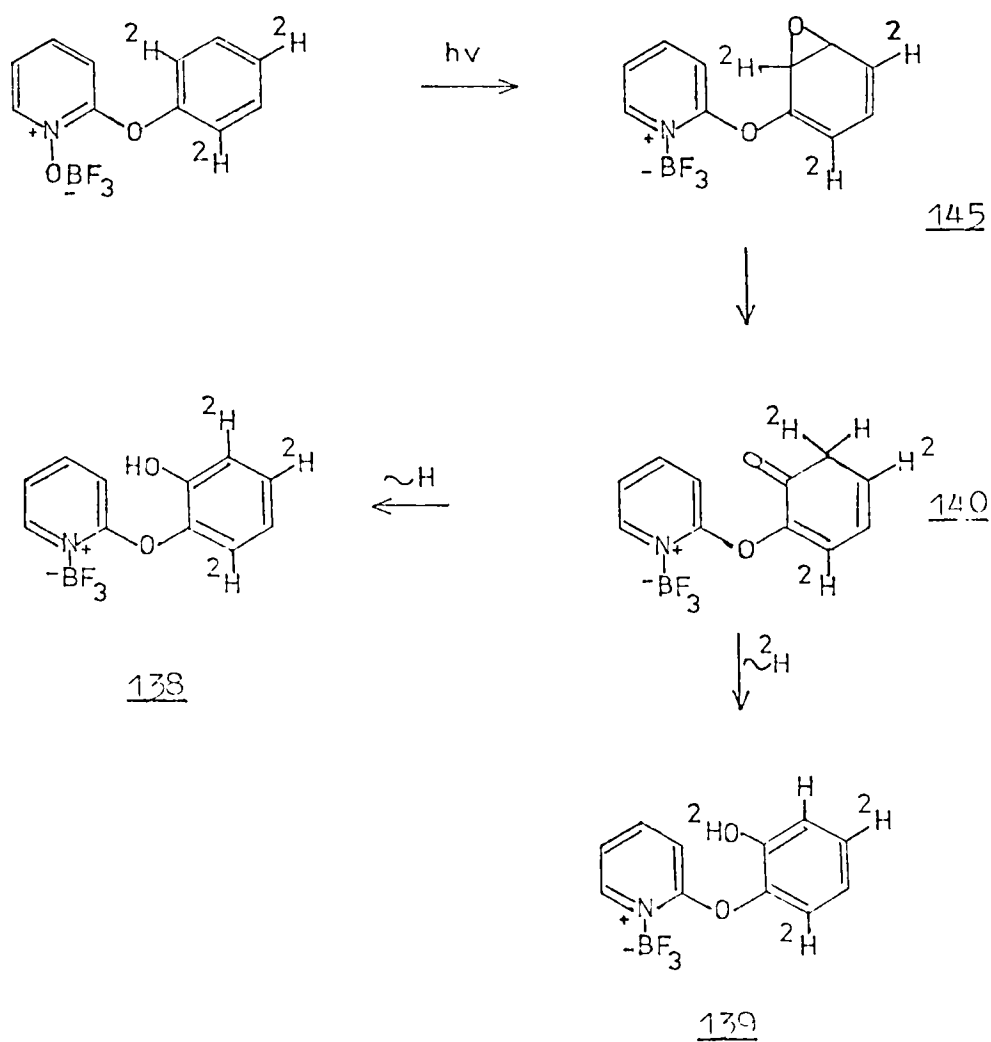
spectroscopy gave m/e 186 (loss of hydrogen from the ^{16}O species 124, 1.97%) and m/e 188 (loss of hydrogen from the ^{18}O labelled species 131, 0.24%). These data indicated the amount of ^{18}O labelled compound 131 to be $\approx 11\%$, below that expected ($\approx 21\%$). However peaks of low intensity do not give reliable results for quantitative essays.

A mixture of the $[\text{}^{18}\text{O}]$ -N-labelled N-oxide 131 (that is a mixture of 124 and 131) and of the deuterated one 129 was converted into their boron trifluoride adducts, irradiated in dichloromethane at 320 nm and separated by both preparative t.l.c. and h.p.l.c. techniques. The purification of the *o*-hydroxylated compound did not present any problem. Complete purification of even small quantities of the *m*- and *p*- isomers proved difficult. For the *o*-hydroxylated product no peak at m/e 192 could be detected : such a species, containing three deuterium (the irradiation of 131 occurred with the NIH shift !) and one ^{18}O atoms should be produced by an intermolecular mechanism and with the NIH shift. Under the operating conditions used, the molecular ion of the natural, unlabelled 2-(2'-hydroxyphenoxy)pyridine 127 had an intensity of 31.64% (m/e 187). Thus, within the experimental limits of high resolution mass spectrometry, the *o*-hydroxylation mechanism appears to be intramolecular.

When Jerina irradiated $[4\text{-}^2\text{H}]$ -anisole with pyridine N-oxide (17), he observed a 45% deuterium retention in the obtained *p*-hydroxyanisole at 25°C (60% at -78°C). The *o*-hydroxylation of 2-([2',4',6'- $^2\text{H}_3$] phenoxy)pyridine N-oxide 131 gave in our hands a 40% retention of ^2H . The *o*-hydroxylation appears to proceed intramolecularly and

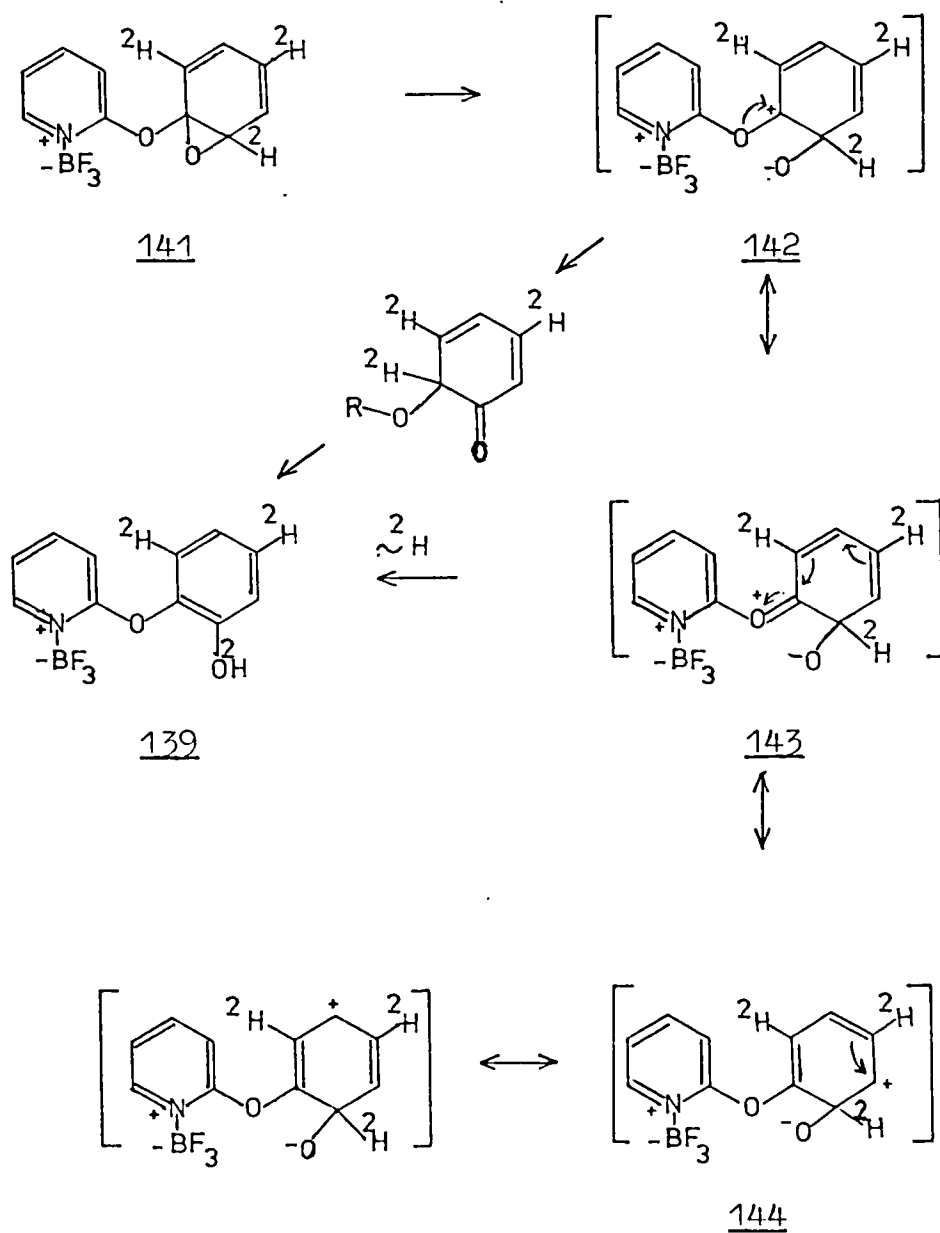
with the formation of arene oxides. It should be interesting to know whether the boron trifluoride intervenes during the hydroxylation process or whether it is blocked by the pyridine. The product 138, obtained with retention of deuterium, could be formed via the mechanism shown in Fig. 72.

Fig. 72



The product 139 can be obtained by loss of deuterium in aromatising from 140, or by an alternative mechanism, from the arene oxide 141 (Fig. 73).

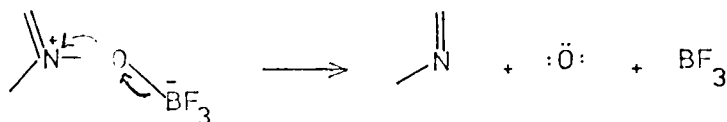
Fig. 73



The intermediate 142, stabilised by its mesomeric structures 143, 144, etc., can aromatise to 130 via HII shift or by loss of ^2H (via 143).

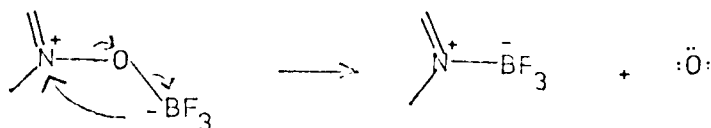
The considerable amount of 139 formed ($\approx 50\%$) with respect to 132 ($\approx 40\%$) suggests that both mechanisms operate. The arene oxide 145 could be formed from the species 144. These mechanisms assume the boron trifluoride to be blocked by the nitrogen, thus excluding it from the aromatisation mechanism. Such a fact could be argued, yet not much is known about the deoxygenation mechanism. The absence of the rearrangement products (derived from an oxaziridine type intermediate) seems to exclude a boron-oxygen cleavage before the nitrogen-oxygen bond breaking. A process of the type shown in Fig. 74 could operate.

Fig. 74



A concerted mechanism could also be assumed (Fig. 75).

Fig. 75



The lone pair of the nitrogen should be blocked by the boron trifluoride with the simultaneous liberation of atomic oxygen.

The oxene should attack the aromatic ring and form the arene oxide 141, which readily aromatises to the phenol 139. An oxygen walk, via 142, 144, could give the arene oxide 145, which aromatises giving the phenols 138 (with NIH shift) and 139. The formation of the arene oxide 141 before its other isomers can be supported by assuming the high regioselectivity of this reaction in general. The adducts of 2-phenethylpyridine N-oxide 105 and 2-benzylpyridine N-oxide 106 afforded 43% of o-hydroxylation (from 106) and 15% (from 105), 15% of benzylic oxidation product 122 (from 105) and 4% of 123 (from 106).

The o-/m-/p- product distribution obtained in irradiating the complex of 124 suggests an electrophilic attack :the species generated also gives benzylic attack and appears very sensitive with respect to stereochemical factors.

This chemical behaviour of the oxidising species could well be explained by the oxene formation. Perhaps low temperature experiments, combined with high resolution spectroscopic techniques, could definitely solve this problem.

EXPERIMENTAL

All melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

Infra-red (i.r.) spectra were recorded for carbon tetrachloride solutions on sodium chloride cells, using a Perkin Elmer 157G spectrophotometer.

Ultra-violet (u.v.) spectra were recorded for methanolic solutions in silica cells, unless otherwise stated, with a Pye-Unicam SP 800 instrument. The data provides λ_{max} values in nanometers (nm).

Nuclear magnetic resonance (n.m.r.) spectra were recorded, unless stated to the contrary, for solutions in deuteriochloroform (CDCl_3), with tetramethylsilane (TMS) as internal standard. For routine work a Varian T 60 instrument was used. For 100 MHz spectra a Jeol NMR 100MH instrument was used. The following abbreviations are used in connection with n.m.r. data : s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet, b = broadened. Chemical shifts are quoted in δ , and coupling constants as J values, in Hz.

Mass spectra were recorded on an AEI MS9 spectrometer. High resolution mass spectra were recorded at P.C.M.U. (Physico-Chemical Measurements Unit), Harwell, Berkshire.

Thin layer chromatography (t.l.c.) was carried out

on Kieselgel GF₂₅₄ (Merck) grade silica gel, activated at 110⁰ C for 1 hour before use. Unless otherwise indicated, chromatograms were developed in chloroform-methanol (2-8% methanol) and visualised using an u.v. source and iodine vapour. Preparative t.l.c. was carried out on plates of 1 mm thickness, under similar conditions. The bands were extracted with methanol ; the solutions were evaporated, treated with chloroform (unless otherwise indicated), filtered and evaporated. All the filtrations were effected through sintered glass funnels.

Solvent mixtures are described in ratios of volumes.

Tetrahydrofuran (THF), diethyl ether (ether) and benzene were dried over and distilled from lithium aluminium hydride (LAH) immediately before use. Toluene and dimethylformamide (DMF) were distilled after drying over calcium hydride, and kept over 4A molecular sieves. Dichloromethane was dried over and distilled from phosphorus pentoxide immediately before use. Petroleum refers to the fraction of b.p. 60-80⁰ C ; it was dried over and distilled from sodium immediately before use, when necessary.

Photolyses were carried out in Pyrex, λ 300 nm, or, if stated, silica (quartz), λ 220 nm, vessels, equipped with glass sinters through which dry, O₂ free nitrogen was passed to expel and exclude oxygen from the solutions, for at least 20 minutes prior to and continuously during irradiation. Solutions were cooled internally at 25-30⁰ C using a

"cold-finger" apparatus. When stated, a Reading reactor was used ; its internal walls are swept by a spinning cleaner, thus preventing the reaction products and tars to stick on them.

Quickfit apparatus was used throughout the work. Stirring was effected with a magnetic stirrer-hotplate, with a follower in the reaction flask, with a silicone oil bath for small reactions. For larger reactions heating mantles with mechanical stirring were used. Reactions in sealed glass tubes were effected in silicone oil baths.

The high pressure liquid chromatography (h.p.l.c.) system employed consisted of a Waters Associates Chromatography Pump (model M 6000 A), of Corasil II and Porasil T columns, and of a Cecil CE 2I2 Linear Readout Ultraviolet Spectrophotometer.

1. Irradiation of pyridine N-oxide in benzene (320 nm)

Pyridine N-oxide (237 mg, commercial product, dried and then purified by vacuum distillation) was dissolved in benzene (40 ml, Analar). The solution was irradiated for 40 minutes (320 nm). The reaction was monitored by t.l.c. (5, 10, 30, 40 min.). The resultant solution was separated and the reactor rinsed with methanol (3x20 ml). The combined portions were evaporated to small bulk at 20 mmHg pressure and room temperature. A brown oil was obtained. A t.l.c. plate confirmed, inter alia, the formation of a product having the same R_f as phenol. The reaction product and reference phenol showed identical behaviour with respect to both irradiation under u.v. light and response to iodine.

Products having the same R_f as pyridine N-oxide and pyridine respectively were also present. The reaction mixture was purified by preparative t.l.c. (7:3, petroleum: ethyl acetate). The band having the same R_f as reference phenol was extracted with acetone (100 ml, Analar) and the solution was evaporated (20 mmHg pressure, 30°C).

The yellow oil obtained (11.4 mg) appeared to contain phenol from its i.r. spectrum. The i.r. spectrum of reference phenol contained bands at 3610, 3050, 1595, 1510, 1475, 1345, 1265, 1180, 1155, 1075, 1030, 1005, 890 and 640 cm^{-1} . The i.r. spectrum from the reaction showed all these bands, except for the 1345 cm^{-1} one, which was obscured by an intense impurity band. Since other bands were also present, the compound was further purified by preparative t.l.c. to afford an oil (8 mg), which was still impure. An

i.r. analysis indicated the presence of about 30% phenol (2.4 mg, ca 1% yield from pyridine N-oxide).

2. Preparation of the standard graph for the u.v. quantitative analysis of phenol

Four different concentration solutions of phenol (purified by distillation, Analar) in methanol were prepared. Their absorbance values (272 nm) were as follows :

- 1) 1.56×10^{-2} g/l , absorbance 0.276
- 2) 2.28×10^{-2} g/l , absorbance 0.394
- 3) 2.88×10^{-2} g/l , absorbance 0.494
- 4) 4.84×10^{-2} g/l , absorbance 0.890

The least square method was employed for plotting a curve out of these experimental data. Having a set of 2n experimental measurements x_i and y_i , a curve corresponding to the equation $y = ax + b$ can be obtained, where

$$a = \frac{n \sum_i x_i y_i - \sum_i x_i \sum_i y_i}{n \sum_i x_i^2 - (\sum_i x_i)^2} \quad \text{and} \quad b = \frac{\sum_i y_i}{n} - a \frac{\sum_i x_i}{n}$$

x_i were the concentrations, in g/l 10^{-2} , and y_i the absorption values.

The curve $y = 18.9x - 0.03$ was obtained.

3. Irradiation of pyridine N-oxide in benzene (320 nm) ;
quantitative u.v. essay of phenol

A solution of pyridine N-oxide (470 mg) in benzene (50 ml, Analar) was irradiated for 3 hours (320 nm). The solution was collected and the reactor was rinsed with methanol (3x20 ml). The combined portions were concentrated at the spinning band column (20 mmHg pressure, 30°C). Phenol was detected by t.l.c. analysis. The residue was treated with ether (40 ml) and aqueous 1N sodium hydroxide (40 ml). The aqueous portion was separated (no phenol was detected in the organic phase by t.l.c.), acidified with dilute aqueous hydrochloric acid, and repeatedly extracted with ether (6x15 ml). The collected organic layers were dried over sodium sulphate, filtered and concentrated at the spinning band column (20 mmHg pressure, 30°C). An oil was obtained and it was purified by preparative t.l.c. (eluted twice in 7:3 petroleum:ethyl acetate, and once in the usual eluent). The band corresponding to phenol was extracted with methanol, to give a methanolic solution (97.427 g). This was estimated for phenol by u.v. quantitative analysis. The averaged absorbance value was 0.595 : on using the curve $y = 18.9x - 0.03$, an x value of 3.3×10^{-2} g/l was obtained. The total volume was 113.3 ml (the density of methanol being 0.8599 g/ml). The total amount of phenol was 3.7 mg, corresponding to a 0.8% yield.

4. Preparation of 2,4,6-tribromophenol (control experiment)

Phenol (250 mg) was dissolved in benzene (50 ml) and concentrated at the spinning band column (20 mmHg pressure, 30°C). The resulting solution was extracted with aqueous 1N sodium hydroxide (3x10 ml). No phenol could be detected in the organic phase (t.l.c.). The aqueous layer was acidified and the 2,4,6-tribromophenol was prepared according to the standard procedure ⁽⁸⁴⁾. The precipitated product was dissolved by adding 1N aqueous sodium hydroxide (20 ml). The clear solution was washed with dichloromethane (2x10 ml). The aqueous layer was acidified with dilute hydrochloric acid and repeatedly extracted with dichloromethane (5x10 ml). These latter organic layers were dried over sodium sulphate, filtered and evaporated. The colourless solid obtained was dried in vacuo overnight and had m.p. 94.5°C (lit. m.p. 95-96°C) (850 mg, 96% yield). The i.r. and n.m.r. spectra of this compound and of reference 2,4,6-tribromophenol were identical.

5. Irradiation of pyridine N-oxide in benzene (320 nm) ; quantitative essay of phenol via 2,4,6-tribromophenol formation

Pyridine N-oxide (1.2 g) was dissolved in dichloromethane (75 ml) and benzene (75 ml) in the Reading reactor, and irradiated (320 nm) for four hours. The solution was collected and the reactor was rinsed with ether-methanol 9:1 (3x10 ml). The combined portions were concentrated at the spinning band column (20 mmHg pressure, 30°C). Toluene

(15 ml) and aqueous sodium hydroxide (4 g in 20 ml) were added. The aqueous phase was separated. The organic phase was extracted again with 1N aqueous sodium hydroxide (2x10 ml). No phenol could be detected by t.l.c. in the organic phase. The combined aqueous layers were acidified with dilute hydrochloric acid, and bromine was added dropwise at 0°C : a precipitate was formed. The solution was warmed up to 45°C, cooled, basified with 1N aqueous sodium hydroxide (the precipitate dissolved) and washed with dichloromethane (2x10 ml). No tribromophenol could be detected in this organic phase (t.l.c.). The aqueous layer was acidified with dilute hydrochloric acid and a precipitate formed, which dissolved after addition of dichloromethane (15 ml). The aqueous layer was repeatedly extracted with dichloromethane (5x10 ml). The combined organic extracts were dried with sodium sulphate, filtered and evaporated. A solid (80 mg) was obtained, which ran as a mixture on t.l.c.. The reaction mixture was purified by preparative t.l.c.. A colourless solid was obtained (45 mg, 1% yield), which had the same m.p., i.r. and n.m.r. spectra as 2,4,6-tribromophenol reference.

6. Preparation of pyridine N-oxide boron trifluoride adduct

Pyridine N-oxide (1.070 g) was dissolved in chloroform (20 ml, dried over Al_2O_3). Boron trifluoride-diethyl ether (1.45 ml, 1 eq.) was syringed into the reaction flask and a colourless precipitate instantaneously formed. The reaction was allowed to stand for 40 minutes, evaporated and dried in vacuo. The resulting solid (1.84 g, 100% yield)

(m.p. 97°C , lit. ⁽⁶⁹⁾ m.p. $97-98^{\circ}\text{C}$) was insoluble in carbon tetrachloride (difference between pyridine N-oxide and its boron trifluoride adduct) and no i.r. of the reaction product could be obtained in that solvent.

The complex could be decomposed as follows : the adduct (450 mg) was dissolved in chloroform (30 ml, dried over Al_2O_3) and alumina (1.5 g) was added. The mixture was stirred for 2 hours (25°C) and filtered. The solid portion was washed with chloroform (4x5 ml) and the combined solutions were concentrated and checked by t.l.c., against pyridine N-oxide boron trifluoride adduct as reference. No complex remained and free pyridine N-oxide was present. Analogous treatment of the boron trifluoride adducts of 2-benzylpyridine N-oxide, 2-phenethylpyridine N-oxide and 2-phenoxy pyridine N-oxide gave a $\approx 95\%$ recovery of the pure N-oxides.

7. Irradiation of pyridine N-oxide boron trifluoride in benzene (254 nm)

Pyridine N-oxide boron trifluoride adduct (1.050 g) was dissolved in benzene (150 ml, Analar) and dichloromethane (200 ml) in the Reading reactor. The solution was irradiated for 4 hours (254 nm). The solution was collected and the reactor was rinsed with ether-methanol 5:1 (4x20 ml). The combined portions were concentrated at the spinning band column (20 mmHg pressure, 30°C). A t.l.c. plate confirmed the formation of phenol. The t.l.c. pattern looked much cleaner than in the analogous reaction with pyridine N-oxide.

The mixture was stirred at room temperature on alumina (3 g). After filtering and washing the solid portion with dichloromethane (6x10 ml), the reaction mixture was concentrated at the spinning band column (20 mmHg pressure, 30°C). The organic phase was extracted with aqueous sodium hydroxide (3 g in 30 ml). No phenol was found in the organic phase (t.l.c.). The aqueous layer was acidified with dilute hydrochloric acid and bromine was added at 0°C, a precipitate immediately forming. The solution was warmed up to 45°C, cooled, treated with 1N sodium hydroxide (the precipitate dissolved) and washed with dichloromethane (2x10 ml). No 2,4,6-tribromophenol was detected in the organic phase (t.l.c.). The aqueous portion was acidified with dilute hydrochloric acid - the precipitate formed again. After dichloromethane addition (15 ml) the precipitate dissolved. The aqueous phase was extracted with dichloromethane (4x10 ml) and the combined organic layers were dried over sodium sulphate, filtered and evaporated. A brown solid (190 mg) was obtained, successively purified by preparative t.l.c. A colourless solid (145 mg, 6.7% yield) resulted, m.p. 93°C ; its i.r. and n.m.r. spectra are identical with those of 2,4,6-tribromophenol reference.

8. Irradiation of pyridine N-oxide boron trifluoride in anisole (254 nm)

Pyridine N-oxide boron trifluoride adduct (1060 mg) was dissolved in anisole (100ml). The solution turned dark violet after addition of the first amount of anisole and

trials to detect charge-transfer complex formation by u.v. spectroscopy failed. The mixture was irradiated in the Reading reactor (254 nm) for 3 hours. The solution was collected and the reactor was rinsed with ether-methanol 9:1 (6x15 ml). The combined portions were concentrated and extracted with 1N aqueous sodium hydroxide (4x20 ml). The aqueous layer was acidified with dilute hydrochloric acid and repeatedly extracted with ether (6x20 ml). The organic layers were dried over sodium sulphate, filtered and evaporated. A yellow oil (95 mg) was obtained after drying in vacuo, further purified by preparative t.l.c. (dichloromethane as eluent). Two compounds were isolated : a yellow oil (55 mg) was obtained and its R_f , i.r. and n.m.r. spectra were identical with those of 2-methoxyphenol (6.9% yield) ; the second compound obtained (3.5 mg) was a yellow oil and its i.r. spectrum contained all the bands of phenol, with the same relative intensities and shapes. The amount of phenol in the oil was estimated to be about 50%.

9. Preparation of 2-phenethylpyridine 107 (70)

A solution of sodamide was prepared from clean sodium metal (10.2 g) in liquid ammonia (350 ml) (85). 2-Methylpyridine (43 ml) was added dropwise and the solution turned red. After addition, benzyl chloride (51 ml) was added as quickly as possible (5 minutes). The ammonia was evaporated and water (200 ml) and dichloromethane (200 ml) were added. The organic phase was separated and the aqueous phase was

repeatedly extracted with dichloromethane (6x50 ml). The collected organic layers were dried over sodium sulphate, filtered and evaporated. A brown oil was obtained (75 g). The mixture (t.l.c.) was purified by vacuum distillation (1 mmHg) and the heads (50-70°C) and the tails (120-135°C) were discarded. The fraction in the middle (110°C/ 1 mmHg) was obtained as a colourless oil (50 g, 63% yield) chromatographically pure (t.l.c.) and appeared to be 2-phenethylpyridine from its n.m.r. spectrum : δ 3.1 (s, 4H, benzylic protons), 7.0-7.7 (m, 8H, aromatic protons), 8.5-8.7 (m, 1H, aromatic proton α to the nitrogen).

10. Preparation of 2-phenethylpyridine N-oxide 105

The procedure of Ochiai (71) was followed :

2-Phenethylpyridine (30 g), glacial acetic acid (200 ml) and 28% aqueous hydrogen peroxide (20 ml, 1 eq.) were stirred at 80°C for 1 hour. Hydrogen peroxide (10 ml) was added again and after 3 hours a more polar product was formed (t.l.c.) almost quantitatively. The reaction was cooled and manganese dioxide (1 g) was added. The mixture was filtered and concentrated (20 mmHg pressure, 60°C). An aqueous solution of sodium hydroxide (20 g in 70 ml) and dichloromethane (100 ml) were added. The organic phase was separated and the aqueous layer was repeatedly extracted with dichloromethane (5x30 ml). The combined organic extracts were washed with aqueous sodium chloride, dried over sodium sulphate, filtered and evaporated. The resulting solid was triturated with petroleum and recrystallised from toluene.

Colourless needles (25 g, 76% yield) were obtained, m.p. 80.5-81°C, $\nu_{\max}$ (CCl₄) 1255 cm⁻¹ (broad and intense, ν_{NO}), $\lambda_{\max}$ (CHCl₃) 273 nm (ϵ = 14930), δ 3.16 (s, 4H, benzylic protons), 7.1-7.26 (m, 8H, aromatic protons), 8.16-8.46 (m, 1H, aromatic proton α to the nitrogen), M/e 199 (M⁺), 183 (M⁺ - O), 182 (M⁺-OH, 100%), 106 and 92 (these peaks suggest a rearrangement, possibly to 110, before a benzylic cleavage). (Found : C 78.37 ; H 6.44 ; N 7.11%. C₁₂H₁₁NO requires : C 78.41 ; H 6.53 ; N 7.02%).

11. Preparation of 2-benzylpyridine N-oxide 106 (72)

2-Benzylpyridine (25 g), 30% aqueous hydrogen peroxide (34 ml) and glacial acetic acid (90 ml) were stirred at 70-80°C for 24 hours. The volatile material was evaporated (20 mmHg pressure, 65°C) and the residue, dissolved in hot chloroform (70 ml), was digested for 10 minutes over anhydrous potassium carbonate (25 g). The mixture was filtered, evaporated and dried in vacuo. The solid which was obtained was triturated with petroleum (20 ml) and recrystallised from toluene. Colourless prisms (24 g, 87%) were obtained, m.p. 100°C (lit. m.p. 100.5°C), $\nu_{\max}$ 1270 cm⁻¹ (broad and intense band, ν_{NO}), $\lambda_{\max}$ (CHCl₃) 272 nm (ϵ =14000), δ 4.2 (s, 2H, benzylic protons), 6.8-7.2 (m, 8H, aromatic protons), 7.9-8.1 (m, 1H, aromatic proton α to the nitrogen), M/e 185 (M⁺), 169 (M⁺-O, plus isotopic peak of M/e 168), 168 (M⁺-OH, 100%), 167 (M⁺-H₂O).

12. Preparation of 2-methoxybenzoic acid methyl ester 113

Commercial 2-methoxybenzoic acid (10 g) was added to a Fisher solution (86). The mixture was refluxed for 1 hour. A less polar product was formed quantitatively (t.l.c.). On evaporation an oil was obtained and to this was added a saturated solution of sodium bicarbonate (20 ml). The mixture was repeatedly extracted with dichloromethane (3x15 ml). The collected organic extracts were dried over sodium sulphate, filtered and evaporated. The resulting product was purified by vacuum distillation. A colourless oil was obtained (9.2 g, 84%), b.p. 85°C/0.7 mmHg), δ 3.93 (s, 6H, methoxy groups), 6.86-8.13 (m, 4H, aromatic protons).

13. Preparation of 2-methoxybenzyl alcohol 114

LAH (1059 mg) was suspended in THF (70 ml) and 2-methoxybenzoic acid methyl ester (8.9 g) in THF (20 ml) was added dropwise at 0°C and under nitrogen, over a 10 minute period. The cooling bath was removed and the mixture was refluxed for 1 hour. A t.l.c. plate confirmed the quantitative formation of a more polar product. The reaction mixture was cooled ; water (1.3 ml), 15% aqueous sodium hydroxide (1.3 ml) and water again (3.9 ml) were successively added, a white precipitate of inorganic salts immediately forming (87). After filtration and repeated washing of the solid portion with dichloromethane (4x10 ml), the collected organic layers were dried over sodium sulphate, filtered and evaporated. A colourless oil (69 g, 90%) was obtained, n_D^{20} 1.548 (lit. n_D^{20} 1.549).

14. Preparation of 2-methoxybenzyl chloride 115

2-Methoxybenzyl alcohol (3.9 g) was dissolved in dichloromethane (30 ml, filtered through alumina) and the reaction flask was protected with a calcium chloride drying tube. Thionyl chloride (2.5 ml, 1.2 eq.) was added and the mixture was refluxed for 2 hours. A t.l.c. plate showed a single product, less polar than the starting material. Aqueous sodium bicarbonate (30 ml) was added and the organic phase was quickly separated, dried over sodium sulphate, filtered and evaporated. A red oil was obtained, which was purified by vacuum distillation. A t.l.c. pure, colourless oil was obtained (2.83 g, 63%), b.p. 85°C/1.8 mmHg, δ 3.86 (s, 3H, methoxy group), 4.66 (s, 2H, benzylic protons), 6.8-7.6 (m, 4H, aromatic protons).

15. Preparation of 2-(2'-o-methoxyphenyl)ethylpyridine 116

Methylolithium (8 ml of a commercial 2.3M solution in hexane, 18.4 mmoles) was syringed into a flask which contained ether (20 ml), at -70°C and under an atmosphere of nitrogen. 2-Methylpyridine (1.71 g), diluted in ether (5 ml) was added with a syringe over a 10 minute period. 2-Methoxybenzyl chloride (2.82 g) in ether (5 ml) was quickly added to the reaction mixture and the solution was allowed to warm up overnight. A major product was formed (t.l.c.) and small amounts of the starting materials were still present. Water (30 ml) was added and the inorganic phase was repeatedly extracted with ether (5x30 ml). The collected organic extracts were dried over sodium sulphate,

filtered and evaporated. A yellow oil (2.1 g) was obtained. Part of this oil (200 mg) was purified by preparative t.l.c. and a t.l.c. pure product was obtained as a yellow oil (154 mg, 41.5%), δ 3.08 (s, 4H, benzylic protons), 3.81 (s, 3H, methoxy group), 8.5-8.76 (m, 1H, aromatic proton α to the nitrogen).

16. Preparation of 2-(2'-o-hydroxyphenyl)ethylpyridine 110 (73)

2-(2'-o-methoxyphenyl)ethylpyridine (154 mg) was dissolved in dichloromethane (20 ml, filtered through alumina). The system was cooled to -70°C , under nitrogen. Boron tribromide (470 mg, 2.3 eq.) dissolved in dichloromethane (10 ml) was added and the mixture was stirred and allowed to reach room temperature overnight. A t.l.c. plate showed the quantitative formation of a more polar product. An aqueous solution of sodium bicarbonate (20 ml) was added and the mixture was stirred for 30 minutes. The aqueous layer was separated and repeatedly extracted with dichloromethane (4x20 ml). The collected organic extracts were combined, dried over sodium sulphate, filtered and concentrated. A yellow oil (124 mg) was obtained after drying in vacuo. Purification by preparative t.l.c. afforded some colourless crystals (80 mg, 56%), m.p. $89.5-90^{\circ}\text{C}$, ν_{max} 3090, 2940, 2850, 2780, 2715, 2625 (broad band system between $3500-2300\text{ cm}^{-1}$, due to the chelated OH ⁽⁷⁴⁾), 1240 (ν phenolic C-O), δ 3-3.5 (m, 4H, benzylic protons), 8.46-8.66 (m, 1H, aromatic proton α to the nitrogen), 9.45 (b, 1H, exchangeable with D_2O , phenolic proton), N/e 199 (M^+ , 63%), 198 (M^+-H , 15%),

187 (M^+-OH , 26%), 93 and 106 (benzylic fragmentation, 100% and 24%). (Found : C 78.16 ; H 6.55 ; N 6.96%. $C_{13}H_{13}NO$ requires : C 78.40 ; H 6.53 ; N 7.02%).

17. Preparation of 2- α -hydroxy- α -(2'-methoxyphenyl)methylpyridine 119

Dry magnesium (340 mg) was covered with THF (3 ml) under nitrogen. 2-Methoxybromobenzene (a few drops) was added and after gentle heating the magnesium started reacting. THF (15 ml) was added. 2-Methoxybromobenzene (total amount 2 g) in THF (15 ml) was added dropwise at room temperature over a 45 minute period. After addition the solution was refluxed (15 minutes) and successively cooled to $0^\circ C$. 2-Formylpyridine (1.1 g) in THF (10 ml) was added dropwise and the reaction was stirred overnight at room temperature. The reaction mixture was treated with dilute aqueous hydrochloric acid, until acidic pH and clear solution. Aqueous sodium hydroxide was added, the precipitate was filtered and the THF evaporated. The aqueous phase was repeatedly extracted with chloroform (5x20 ml). The organic extracts were dried over sodium sulphate, filtered and evaporated. A brown oil (2.2 g) was obtained, which gave a major spot on t.l.c., more polar than both starting materials. The crude reaction product was purified by vacuum distillation. A chromatographically pure (t.l.c.) oil was obtained (1.7 g, 75%), b.p. $90^\circ C/1$ mmHg, ν_{max} 3410, 3070, 3010, 2960, 2940, 1595, 1493, 1468, 1440, 1405, 1240, 1049 cm^{-1} , δ 3.9 (s, 3H, methoxy group), 5.1 (b, 1H, exchanges with D_2O , alcoholic

proton), 6.26 (b, 1H, benzylic proton), 6.76-7.86 (m, 7H, aromatic protons), 8.53-8.73 (m, 1H, aromatic proton α to the nitrogen).

18. Preparation of 2-(2'-methoxyphenyl)methylpyridine 120

2- α -Hydroxy- α -(2'-methoxyphenyl)methylpyridine (120 mg) in ether (5 ml) was syringed into a solution of methyllithium in ether (0.25 ml of a 2.3M commercial solution in hexane, in 10 ml), at 0°C and under nitrogen. Tosyl chloride (114 mg) in ether (5 ml) was added and the reaction mixture was stirred at 30°C for 1 hour and then refluxed for 40 minutes. A major product was formed (t.l.c.). LAH (20 mg) was added after cooling, and the reaction mixture was then refluxed for 1 hour. Water (10 ml) was added and the solution was acidified with dilute hydrochloric acid, basified with 1N aqueous sodium hydroxide and filtered. The solid was washed with ether (3x10 ml) and the combined organic extracts were dried over sodium sulphate and concentrated. A mixture of three components (t.l.c.) was obtained as a yellow oil (160 mg). The oil was purified by preparative t.l.c. and the three products were isolated and examined by n.m.r.. 2-(2'-Methoxyphenyl)methylpyridine was obtained as a yellow oil (35 mg, 31%), δ 3.85 (s, 3H, methoxy group), 4.21 (s, 2H, benzylic protons), 6.6-7.68 (m, 7H, aromatic protons), 8.3-8.63 (m, 1H, aromatic proton α to the nitrogen).

19. Preparation of 2-(2'-hydroxyphenyl)methylpyridine 111

2-(2'-Methoxyphenyl)methylpyridine (35 mg) was dissolved in dichloromethane (5 ml, filtered through alumina) under nitrogen. The mixture was cooled to -70°C and boron tribromide (125 mg) in dichloromethane (3 ml, filtered through alumina) was added. The mixture was allowed to reach room temperature with stirring overnight. Aqueous sodium bicarbonate (5 ml) was added and the organic phase was separated. The inorganic phase was repeatedly extracted with chloroform (3x10 ml). The combined organic layers were dried over sodium sulphate, filtered and evaporated. A solid product, more polar than the starting material (t.l.c.), was obtained. The compound was purified by preparative t.l.c. and colourless crystals (25 mg, 72%) were obtained, m.p. 96.5°C ; the spectral and microanalytical data confirmed the structure:

ν_{max} 3150, 3080, 3040, 2970, 2870, 2590, 2540, 2460 (broad system of bands, extending from 3500 to 2400 cm^{-1} , due to the chelated OH ⁽⁷⁴⁾), 1305, 1270, 1260 (ν phenolic C-O) cm^{-1} , δ 4.11 (s, 2H, benzylic protons), 6.66-7.6 (m, 7H, aromatic protons), 8.4-8.6 (m, 1H, aromatic proton α to the nitrogen), 11.5 (b, 1H, exchangeable with D_2O , phenolic proton), M/e 185 (M^+ , 45%), 184 ($\text{M}^+ - \text{H}$, 15%), 168 ($\text{M}^+ - \text{OH}$, 100%). (Found : C 77.52 ; H 5.97 ; N 7.65%. $\text{C}_{12}\text{H}_{11}\text{NO}$ requires : C 77.85 ; H 5.94 ; N 7.56%).

20. Irradiation of 2-phenethylpyridine N-oxide in dichloromethane (320 nm)

2-Phenethylpyridine N-oxide (184 mg) was dissolved in dichloromethane (40 ml) and irradiated at 320 nm for 2 hours. The reaction was monitored by t.l.c. at intervals. A complex mixture was obtained. After concentration of the reaction mixture a brown oil was obtained and it was purified by preparative t.l.c. . Three successive purifications were carried out and seven fractions were isolated (in order of decreasing polarity) : I, red solid ; II, oil (18 mg); III, solid, starting material (14 mg) ; IV, solid (30 mg); V, oil, 2-phenethylpyridine (31 mg) ; VI, oil (7 mg) ; VII, oil (11 mg).

Compounds III and V corresponded to 2-phenethylpyridine N-oxide 105 and 2-phenethylpyridine 107 respectively. They were reasonably pure on t.l.c. and had coincident R_f values with the reference compounds ; their structures were definitely assigned by i.r. and n.m.r. spectral data. The compound IV needed further purification for identification; two successive purifications (t.l.c.) afforded some reasonably pure crystals (t.l.c.) (12 mg) with m.p. 80°C . I.r., n.m.r. and mass spectral data suggested it to be 2-formyl-5-phenethylpyrrole 121 : ν_{max} 3280 (NH), 1660 (C=O) cm^{-1} , δ 3.02 (s, 4H, ethylenic bridge), 6.06-6.16 (m, 1H, pyrrolic proton),* 7.1-7.43 (m, 5H, aromatic protons), 9.4 (s, 1H, aldehydic proton), 12 (b, 1H, exchangeable with D_2O , N-H proton), M/e 199 (M^+ , 74%), 170 ($\text{M}^+ - \text{CHO}$, 25%), 103 and 91 (benzylic break, 100% and 21%).

* 6.9-7 (m, 1H, pyrrolic proton)

Products I, II, VI and VII were not 2-(2'-o-hydroxyphenyl)ethylpyridine 110 or other aromatic hydroxylation products. All their mass spectra showed a peak at M/e 199 and those at M/e 91 and 108 were also present, suggesting molecules with non-hydroxylated benzene rings. All their i.r. spectra showed intense carbonyl absorptions.

Analogous experiments carried out at 254 nm gave similar results.

21. Irradiation of 2-phenethylpyridine N-oxide 105 (254 nm); 2-(2'-o-hydroxyphenyl)ethylpyridine 110 reference is used in the purification procedure

2-Phenethylpyridine N-oxide (257 mg) in dichloromethane (50 ml) was irradiated for 45 minutes at 254 nm. A brown oil was obtained on concentrating (260 mg), purified by preparative t.l.c.. 2-(2'-o-hydroxyphenyl)ethylpyridine was run as a reference and the band having its R_f was separated and afforded a compound which was again purified on t.l.c. : two purifications afforded colourless crystals (1.6 mg, 0.62% yield), m.p. 89°C. Its i.r. and mass spectral data were identical to the corresponding spectra of the reference compound 110.

22. Irradiation of 2-phenethylpyridine N-oxide 105 in benzene (254 nm)

2-Phenethylpyridine N-oxide (201 mg) was dissolved in benzene (50 ml, Analar) and irradiated at 254 nm for

50 minutes. The reaction mixture was concentrated at the spinning band column (20 mmHg pressure, 30°C). A t.l.c. plate showed a complex mixture : a compound having the same R_f as phenol was present (such a compound was not present in the irradiations of 2-phenethylpyridine N-oxide in dichloromethane). The mixture was purified by preparative t.l.c. and the bands corresponding to phenol, 2-phenethylpyridine, 2-(2'-o-hydroxyphenyl)ethylpyridine and 2-phenethylpyridine N-oxide were extracted. Workup afforded reasonably pure starting N-oxide (48 mg, 24% recovery) and 2-phenethylpyridine (19 mg, 10% yield). The other products required further purifications. The isolation of eventual 2-(2'-o-hydroxyphenyl)ethylpyridine failed (the purification was carried out with the help of reference compound 110), whilst a mixture containing phenol was isolated. The presence of phenol was evidenced by an i.r. spectrum ; the bands at $\nu_{\max}$ 3600, 3070, 1600, 1470, 1270, 1160, 1080, 1040, 1010, 990 and 700 cm^{-1} were present, with the same relative intensity and shape of those in the i.r. spectrum of reference phenol.

23. Irradiation of 2-benzylpyridine N-oxide 106 (254 nm)

2-Benzylpyridine N-oxide (257 mg) in dichloromethane (50 ml) was irradiated at 254 nm for 2½ hours and the reaction was frequently monitored by t.l.c.. A complex mixture was obtained. The reaction mixture was concentrated and purified by preparative t.l.c. with 2-(2'-hydroxyphenyl)-methylpyridine 111 as a reference. The plates were eluted

twice with 4:1 petroleum-ethyl acetate, and twice with the usual eluent. The band having the same R_f as the reference compound 111 afforded a product which required further purification, effected by preparative t.l.c. Colourless crystals were finally obtained (10 mg, 4% yield), m.p. 96°C . The i.r. and n.m.r. spectral data confirmed the product to be 2-(2'-hydroxyphenyl)methylpyridine 111. The other compounds isolated were 2-benzylpyridine 109 (11 mg, 5%), identified by comparison with a reference sample, and a rearrangement product (23 mg, 9.6% yield). The i.r. and n.m.r. data of the latter suggested it to be 2-formyl-5-benzylpyrrole : ν_{max} 3280 (NH) and 1640 (CO) cm^{-1} , δ 4.03 (s, 2H, benzylic protons), 6.1-6.37 (m, 1H, pyrrole proton), 6.9-7.05 (1H, pyrrole proton), 7.26 (b, 5H, aromatic protons), 9.43 (s, 1H, aldehydic proton), 12.5 (b, 1H, exchangeable with D_2O , N-H proton). Both spectra showed close analogies with the corresponding ones of 2-formyl-5-phenethylpyrrole 121.

24. Preparation of 2-phenethylpyridine N-oxide boron trifluoride

2-Phenethylpyridine N-oxide (343 mg) was dissolved in chloroform (10 ml, filtered through alumina) and gaseous boron trifluoride was bubbled through the solution. Colourless crystals gradually formed (1 hour). The formation of the complex was checked by i.r. spectroscopy ; an i.r. (CHCl_3) spectrum showed the lowering of the NO stretching band (1160 cm^{-1} ; 1260 cm^{-1} in the starting material 105).

Evaporation and drying in vacuo afforded colourless crystals (458 mg, 100% yield), m.p. 113°C , λ_{max} 273 nm ($\epsilon=7540$), δ 2.9-3.18 (m, 2H, benzylic protons), 3.3-3.6 (m, 2H, benzylic protons), 6.9-8.0 (m, 8H, aromatic protons), 8.5-8.65 (m, 1H, aromatic proton α to the nitrogen).

25. Irradiation of the boron trifluoride complex of 2-phenethylpyridine N-oxide (254 nm)

2-Phenethylpyridine N-oxide boron trifluoride (687 mg) in dichloromethane (100 ml) was irradiated at 254 nm for 4 hours. The reaction mixture was pipetted out and the reactor was rinsed with methanol (6x30 ml). The combined portions were evaporated and the resulting brown oil was dissolved in chloroform (40 ml), stirred over alumina (1.5 g) and filtered. The mixture was dried over sodium sulphate, filtered and concentrated. The crude reaction mixture gave a clean t.l.c. pattern, only four products being detected by t.l.c. (iodine and u.v.). The mixture was charcoaled in methanol overnight. After filtering, evaporating and drying in vacuo, a solid was obtained (488 mg). The mixture was purified by preparative t.l.c. (4:1 petroleum-ethyl acetate, eluted twice, and usual eluent, eluted twice). Four bands were separated and afforded the following compounds, in order of decreasing polarity : I, solid, 2-phenethylpyridine N-oxide 105 (347 mg, 64% recovery from the starting material); II, solid, m.p. 110°C (34.7 mg) ; III, oil (36.5 mg); IV, oil, 2-phenethylpyridine 107 (28.8 mg, 19% yield).

Compound III had identical R_f than 2-(2'-o-hydroxyphenyl)ethylpyridine 110 and needed further purification ; a solid (26 mg, 15.7% yield) was obtained and it had m.p. 89°C , with identical i.r. and n.m.r. spectral properties to 2-(2'-o-hydroxyphenyl)ethylpyridine 110.

Compound II was further purified by preparative t.l.c. and a solid (25.6 mg, 15.4% yield) was obtained, isomeric with the starting material (mass spectrum) ; its i.r., n.m.r. and mass spectra suggested it to be 2-(2'-hydroxy-2'-phenyl)-ethylpyridine 122 : ν_{max} 3635, 3480, 3100, 3080, 3040, 2920, 1600, 1478, 1440, 1340, 1285, 1210, 1155, 1110, 1060, 1035, 920, 900, 880, 700 cm^{-1} , δ 3.1-3.2 (d, $J=6\text{ Hz}$, 2H, coupled with the triplet at δ 5.2, benzylic protons), 5.2 (t, $J=6\text{ Hz}$, 1H, coupled with the doublet at δ 3.1-3.2, benzylic proton), 5.59 (b, 1H, exchangeable with D_2O , alcoholic proton), 6.86-7.8 (m, 8H, aromatic protons), 8.4-8.6 (m, 1H, proton α to the nitrogen), M/e 199 (M^+ , 80%), 198 ($M^+-\text{H}$, 65%), 180 ($M^+-\text{H}_2\text{O}$, 70%), 107 and 92 (benzylic break, 85% and 100% : this fragmentation suggests the hydroxyl group to be α to the phenyl ring).

26. Preparation of 2-(2'-hydroxy-2'-phenyl)ethylpyridine 122

Methylolithium (5 ml of a commercial 2.3M solution in hexane) was syringed into a THF solution (15 ml) at 0°C , under nitrogen. 2-Methylpyridine (1 g) in THF (10 ml) was added dropwise. Benzaldehyde (1.14 g) in THF (8 ml) was added after 20 minutes. A small amount of solution was worked up after 40 minutes and the quantitative formation of a more

polar product was detected (t.l.c.). The solution was evaporated and aqueous dilute hydrochloric acid (10 ml) was added. The solution was neutralised with aqueous sodium bicarbonate and repeatedly extracted with dichloromethane (4x30 ml). The combined organic extracts were dried over sodium sulphate, filtered and evaporated. The solid obtained was recrystallised from hexane-benzene 1:1 to give colourless crystals (1.4 g, 66% yield), m.p. 110°C. This compound had the same R_f , i.r., n.m.r. and mass spectra as the benzyl alcohol isolated from the irradiation of 2-phenethylpyridine N-oxide boron trifluoride in dichloromethane.

27. Irradiation of the boron trifluoride complex of 2-benzylpyridine N-oxide (254 nm)

The boron trifluoride complex of 2-benzylpyridine N-oxide (1.36 g) was prepared with boron trifluoride-diethyl ether. It was obtained as a solid with m.p. 140°C and checked by u.v., i.r. and n.m.r. : $\lambda_{\max}$ 272 nm ($\epsilon=6600$), $\nu_{\max}$ (CHCl_3) 1150 cm^{-1} (the N-O band was shifted from 1265 cm^{-1} in 106 to 1150 cm^{-1} in the adduct), δ 4.4 (s, 2H, benzylic protons), 6.9-8.0 (m, 8H, aromatic protons), 8.5-8.7 (m, 1H, aromatic proton α to the nitrogen).

The complex was dissolved in dichloromethane (400 ml) and irradiated at 254 nm for 5 hours. The reaction mixture was concentrated and the reactor was rinsed with methanol (6x30 ml). The combined portions were evaporated and an oil was obtained. This was dissolved in chloroform (40 ml) and stirred for 1 hour over alumina (3 g). The t.l.c. plate

showed four products, two of them having the same R_f as 2-benzylpyridine N-oxide 106 and 2-benzylpyridine 109, respectively. After filtration and evaporation, a green oil (1.24 g) was obtained. The reaction mixture was purified by column chromatography (150 g of silica, dichloromethane as the eluent, the polarity gradually increased by addition of methanol). The fractions containing the less polar product (main product on t.l.c.) were further purified by preparative t.l.c. and colourless crystals were obtained (0.43 g, 43.6% yield), m.p. 96°C ; its R_f value on t.l.c., and its spectroscopic properties (i.r., n.m.r., mass) confirmed it to be identical to the previously synthesised 2-(2'-hydroxyphenyl)-methylpyridine 111. A preparative t.l.c. purification on the column fractions mostly containing the second product afforded 2-benzylpyridine 109 (70 mg, 7.6% yield), identified by its spectral properties (i.r. and n.m.r.), identical to those of a reference sample. A third solid compound (40 mg, 4% yield), m.p. 82°C , was obtained after preparative t.l.c. on further column fractions. Its n.m.r. spectrum suggested it to be 2-(α -hydroxy- α -phenyl)methylpyridine 123, which was independently synthesised; the m.p. and n.m.r. spectra of both compounds were identical: δ 5.2 (b, 1H, exchangeable with D_2O , alcoholic proton), 5.8 (s, 1H, benzylic proton), 7.0-7.9 (m, 8H, aromatic protons), 8.4-8.6 (m, 1H, aromatic proton α to the nitrogen).

28. Preparation of 2-(α -hydroxy- α -phenyl)methylpyridine 123

The Grignard reagent of bromobenzene (530 mg, 3.37 mmoles) was prepared from magnesium (80 mg, 3.4 mg-atoms) in THF (30 ml) under nitrogen. 2-Formylpyridine (340 mg, 3.2 mmoles) in THF (20 ml) was added and the solution was refluxed for 1 hour. A t.l.c. plate showed the formation of a main more polar product. Aqueous dilute hydrochloric acid was added (15 ml), the solution was neutralised with solid sodium bicarbonate and the THF evaporated. The aqueous portion was repeatedly extracted with dichloromethane (6x20 ml) and the combined organic extracts were dried with sodium sulphate, filtered and evaporated. A solid compound was obtained and recrystallised from toluene (350 mg, 56% yield). The R_f , m.p. (82°C) and n.m.r. spectrum were identical to the ones of the benzylic alcohol, obtained from irradiating the boron trifluoride complex of 2-benzylpyridine N-oxide.

29. Irradiation of 2-benzylpyridine N-oxide in aqueous sulphuric acid (254 nm)

2-Benzylpyridine N-oxide (1.03 g) was dissolved in aqueous sulphuric acid (300 ml, 23 mmoles H_2SO_4 per mmole of N-oxide) and irradiated at 254 nm for 7 hours. The reaction mixture was neutralised with solid sodium bicarbonate and repeatedly extracted with chloroform (7x50 ml). The combined organic layers were collected, dried over sodium sulphate, filtered, evaporated and charcoaled. A yellow oil was obtained, which showed a t.l.c. pattern very similar to the one obtained on irradiating the boron trifluoride complex of 2-benzyl-

pyridine N-oxide ; traces of other products could be seen. The crude reaction mixture was purified by column chromatography (100 g of silica, dichloromethane as the eluent ; the polarity was gradually raised with methanol). The fractions containing the three less polar products were furtherly purified by preparative t.l.c. and three substances were isolated : I, solid, 2-(2'-hydroxyphenyl)methylpyridine 111 (164.3 mg, 15.9%) ; II, oil, 2-benzylpyridine 109 (40.8 mg, 4.3%) ; III, solid, 2-(α -hydroxy- α -phenyl)methylpyridine 123 (30 mg, 3.8%). The compounds I, II and III were identified by comparison with reference samples (R_f , m.p., i.r. and n.m.r. spectra). The presence of a large amount of starting material in the reaction mixture was confirmed by the i.r. and n.m.r. spectra of the crude mixture.

30. Preparation of 2-phenoxy pyridine N-oxide 124

2-Chloropyridine N-oxide 126 (7.82 g, 61 mmoles) and sodium phenate (14.1 g, 136 mmoles) were dissolved in DMF (60 ml). The mixture was stirred at 65°C for 2 hours under nitrogen. A small sample showed the quantitative formation of a more polar product (t.l.c.). Most of the DMF was removed by vacuum distillation (40 mmHg pressure). Aqueous sodium hydroxide (30 g, 0.53 moles in 50 ml) and chloroform (100 ml) were added to the reaction mixture. The organic phase was separated and repeatedly washed with water (2x10 ml). The organic phase was dried over sodium sulphate, filtered and evaporated, a brown solid (11.28 g) being obtained after drying in vacuo. The crude product looked reasonably pure (t.l.c.) and was purified by column chromatography (400 g

of alumina, dichloromethane as eluent ; the polarity was gradually increased with methanol). The fractions containing pure product (t.l.c.) were reunited and evaporated. Colourless crystals were obtained (6.78 g, 60% yield), m.p. 89°C and i.r., n.m.r. and mass spectral data confirmed the product to be the title compound : ν_{max} 3060, 3040, 1590, 1485, 1300, 1275, 1240, 1195, 1160, 1100, 1070, 1020, 890, 685 cm^{-1} , δ 6.76-7.52 (m, 8H, aromatic protons), 8.2-8.38 (m, 1H, aromatic proton α to the nitrogen), M/e 187 (M^+ , 1.88%), 186 ($\text{M}^+ - \text{H}$, 2.2%), 171 ($\text{M}^+ - \text{O}$, 51.3%), 170 ($\text{M}^+ - \text{OH}$, 100%).

31. Preparation of 2-phenoxy pyridine

Sodium phenate (4.1 g, 35 mmoles), phenol (4 g, 43 mmoles) and 2-chloropyridine (3 g, 26.5 mmoles) were sealed in a glass tube, care to avoid moisture being taken. The reaction mixture was warmed at 160°C for 15 hours. Aqueous sodium hydroxide (10 g, 390 mmoles in 40 ml) was added and the reaction mixture was repeatedly extracted with ether (5x20 ml). The collected ethereal extracts were dried over sodium sulphate, filtered and evaporated, a yellow oil being obtained. A t.l.c. plate confirmed the formation of a more polar product. After drying in vacuo overnight a solid was obtained (4.25 g). The compound was purified by vacuum distillation (120°C , 0.5 mmHg pressure, distilled twice). Colourless crystals were obtained (3.67 g, 61% yield) and had m.p. 41°C . The i.r. and n.m.r. spectroscopic data did confirm the formation of 2-phenoxy pyridine : ν_{max} 3050 (broad), 1590, 1490, 1468, 1430, 1285, 1265, 1250, 1204,

880, 790 cm^{-1} , δ 6.6-7.7 (m, 8H, aromatic protons), 8.08-8.22 (m, 1H, aromatic proton α to the nitrogen).

32. Preparation of 2-(2'-methoxyphenoxy)pyridine 130

a) Preparation of the sodium salt of 2-methoxyphenol

2-Methoxyphenol (6.74 g) in THF (20 ml) was added dropwise to a THF (30 ml) suspension of sodium hydride (2.17 g)* at 0°C under nitrogen. The mixture was allowed to warm up to room temperature. After evaporation and drying in vacuo, trituration with petroleum, filtration, evaporation and drying in vacuo, a solid was obtained (7.3 g, 92% yield).

b) Condensation between 2-chloropyridine and the sodium salt of 2-methoxyphenol

The sodium phenate obtained in a) (7.3 g, 50 mmol) and 2-chloropyridine (7 g, 61 mmol) were sealed in a glass tube, care to avoid moisture being taken. The mixture was warmed at 180°C for 10 hours. Aqueous potassium hydroxide (6.2 g, 110 mmol in 50 ml) and dichloromethane (40 ml) were added. The organic phase was separated, dried over sodium sulphate, filtered and evaporated. A less polar product was formed (t.l.c.). The reaction mixture was charcoaled in methanol and the resulting solid was trituated with petroleum, filtered and dried in vacuo. Colourless crystals (4.5 g, 44% yield) were obtained and had m.p. 91°C , δ 3.71 (s, 3H, methoxy group), 6.7-7.21 (m, 6H, aromatic protons), 8.0-8.12 (m, 1H, aromatic proton α to the nitrogen).

* 60% dispersion in min. oil

33. Preparation of 2-(2'-hydroxyphenoxy)pyridine 127

2-(2'-Methoxyphenoxy)pyridine 130 (2.14 g) in chloroform (10 ml, filtered through alumina) was dropped into a chloroform (20 ml) solution of boron tribromide (7.9 g, 3 eq.) at -70°C . The reaction mixture was allowed to warm up to 25°C with stirring overnight, and t.l.c. showed that a single more polar product was formed. Water (25 ml) was added and the resulting acidic solution was neutralised with solid sodium bicarbonate. The solution was filtered and repeatedly extracted with chloroform (8x20 ml). The organic phases were dried over sodium sulphate, filtered and evaporated. A solid was obtained after drying in vacuo (1.68 g, 89% yield). Part of it was recrystallised twice from a 1:5 methanol-toluene mixture. Colourless crystals (300 mg) were obtained, m.p. 110°C . The spectral and microanalytical data confirmed it to be 2-(2'-hydroxyphenoxy)pyridine 127 : ν_{max} 3565, 3040, 2920, 2850 (broad system of bands extending from 3500 to 2500 cm^{-1} , due to the chelated OH ⁽⁷⁴⁾), 1590, 1570, 1490, 1465, 1430, 1290, 1270, 1240, 1205, 1170, 1140, 890 cm^{-1} , δ 6.8-7.3 (m, 6H, aromatic protons), 7.5-7.71 (m, 1H, aromatic), 7.97-8.18 (m, 1H, aromatic proton α to the nitrogen), 8.3 (b, exchangeable with D_2O , 1H, phenolic proton), M/e 187 (M^+ , 30%), 186 ($\text{M}^+ - \text{H}$, 8%), 170 ($\text{M}^+ - \text{OH}$, 100%), 79 ($\text{C}_5\text{H}_5\text{N}^+$, 15%), 78 ($\text{C}_5\text{H}_4\text{N}$, 31%). (Found : C 69.71 ; H 4.89 ; N 7.20%. $\text{C}_{11}\text{H}_9\text{NO}_2$ requires: C 70.56 ; H 4.84 ; N 7.49%).

34. Preparation of 2-(3'-methoxyphenoxy)pyridine 131

a) Preparation of the sodium salt of 3-methoxyphenol

The salt was prepared from 3-methoxyphenol (2.18 g) with an analogous procedure and workup as in the preparation 33, part a). A solid (1.62 g, 63% yield) was obtained.

b) Condensation between 2-chloropyridine and the sodium salt of 3-methoxyphenol

The sodium salt of 3-methoxyphenol (1.62 g, 11.08 mmoles) and 2-chloropyridine (1.6 g, 14.14 mmoles) were reacted with an analogous procedure and workup as in the preparation 33, part b). A yellow oil was obtained and purified by vacuum distillation (b.p. 105° C/0.5 mmHg). A colourless oil was obtained (1.54 g, 69% yield), pure on t.l.c., δ 3.77 (s, 3H, methoxy group), 6.65-7.10 (m, 5H, aromatic protons), 7.16-7.20 (m, 1H, aromatic proton), 7.5-7.72 (m, 1H, aromatic proton), 8.12-8.22 (m, 1H, aromatic proton α to the nitrogen).

35. Preparation of 2-(3'-hydroxyphenoxy)pyridine 128

The reaction was effected between boron tribromide (5.18 g, 3 eq.) and 2-(3'-methoxyphenoxy)pyridine 131 (1.5 g). An analogous procedure and workup as described for the preparation 33 were followed. A solid (1.20 g, 84%), m.p. 156-158° C, was obtained after trituration with petroleum. Part of it was recrystallised from 9:1 chloroform-methanol and colourless crystals were obtained (350 mg), m.p. 158° C, $\nu_{\max}$ 3590, 3290 (very broad), 1590, 1575, 1485, 1470, 1430, 1290, 1160, 1140, 1000, 965, 860 cm^{-1} , M/e 187 (M^+ , 70%),

186 (M^+-H , 100%), 159 (M^+-CO , 45%), 78 ($C_5H_4N^+$, 25%), at 70 eV ; M/e 187 (M^+) and 188 (^{13}C isotopic peak of M^+) only are present in the molecular ion region at 9.5 eV.

36. Preparation of 2-(4'-methoxyphenoxy)pyridine 132

a) Preparation of the sodium salt of 4-methoxyphenol

The sodium salt of 4-methoxyphenol (2.56 g, 20.7 mmols) was prepared with an analogous procedure and workup as in preparation 32, part a).

b) Condensation between 2-chloropyridine and the sodium salt of 4-methoxyphenol

2-Chloropyridine (2 g, 17 mmols) and the sodium salt from part a) of this preparation were reacted and worked up as in preparation 33, part b). After trituration from petroleum, some pure (t.l.c.) crystals were obtained (3.01 g, 87% yield), m.p. $44^{\circ}C$, δ 3.81 (s, 3H, methoxy group), 6.83-7.18 (m, 6H, aromatic protons), 7.58-7.75 (m, 1H, aromatic proton), 8.1-8.23 (m, 1H, aromatic proton α to the nitrogen).

37. Preparation of 2-(4'-hydroxyphenoxy)pyridine 129

2-(4'-Methoxyphenoxy)pyridine 132 (373 mg) and boron tribromide (1.39 g, 3 eq.) were reacted and worked up as in preparation 33. After trituration from petroleum, colourless crystals, pure on t.l.c., were obtained (270 mg, 1.44 mmols, 78% yield), m.p. $137^{\circ}C$. The product was confirmed by spectral and microanalytical data : ν_{max} ($CHCl_3$) 3590, 3300 (broad), 1594, 1582, 1500, 1470, 1425, 1290, 1095, 990, 880 cm^{-1} , δ 6.6-7.0 (m, 6H, aromatic protons), 7.18 (b, s, 1H, phenolic

proton, exchangeable with D_2O), 7.5-7.8 (m, 1H, aromatic proton), 8.0-8.2 (m, 1H, aromatic proton α to the nitrogen), M/e 187 (M^+ , 100%), 186 (M^+-H , 80%), 159 (M^+-CO , 70%), 78 ($C_5H_4N^+$, 35%), at 70 eV; M/e 187 (M^+) and 188 (^{13}C isotopic peak of M^+) only are present in the molecular ion region at 10 eV. (Found : C 69.11 ; H 4.84 ; N 7.30%. $C_{11}H_9NO_2$ requires: C 70.56 ; H 4.84 ; N 7.49%).

38. Preparation of 2-phenoxy-pyridine N-oxide boron trifluoride

The boron trifluoride adduct of 2-phenoxy-pyridine N-oxide (207 mg, 1.1 mmoles) was prepared as in preparation 6. The formation of the complex (282 mg, 100%), m.p. $138^{\circ}C$, was checked by i.r. and n.m.r. : ν_{max} ($CHCl_3$) 3150-3040 (broad), 1670, 1575, 1500, 1483, 1325, 1185, 1160, 1090, 900, 835 cm^{-1} , δ 6.9-8.2 (m, 8H, aromatic protons), 8.5-8.7 (m, 1H, aromatic proton α to the nitrogen).

39. Irradiation of the boron trifluoride complex of 2-phenoxy-pyridine N-oxide (320 nm)

The boron trifluoride complex of 2-phenoxy-pyridine N-oxide (279 mg, 1.09 mmoles) was dissolved in dichloromethane (45 ml) and irradiated (320 nm) at $25^{\circ}C$. The reaction was monitored (t.l.c.) until most starting material reacted (7 hours). A brown oil was obtained after evaporation. The crude reaction mixture was treated with aqueous sodium bicarbonate (30 ml) and chloroform (50 ml). The aqueous phase was continuously extracted and the organic phase was dried over sodium sulphate, filtered and evaporated. A yellow oil

(219 mg) was obtained after charcoaling in methanol and drying in vacuo. Four products could be detected in a clean t.l.c. pattern. The reaction mixture was purified by preparative t.l.c. (eluted twice in 3:1 petroleum-ethyl acetate, and one time in the usual eluent). Four products were obtained : I, oil, 2-phenoxy pyridine (31.1 mg) ; II, solid, 2-(2'-hydroxyphenoxy)pyridine 127 (69.0 mg) ; III, solid, 2-(4'-hydroxyphenoxy)pyridine 129 (14.7 mg); IV, solid, 2-phenoxy pyridine N-oxide 124 (38 mg).

The structures of the four products were determined by comparison with reference samples (I, R_f and i.r. ; II, R_f , m.p., i.r., n.m.r. and mass spectra ; III, R_f , m.p., i.r., n.m.r. and mass spectra ; IV, R_f and i.r. spectrum). The yields obtained were the following (from the reacted N-oxide, 0.89 mmoles) : 2-phenoxy pyridine, 20.3% ; 2-(2'-hydroxyphenoxy)pyridine 127, 41% ; 2-(4'-hydroxyphenoxy)pyridine 129, 7.2% ; overall deoxygenation 68.5%.

40. Preparation of the standard graph for an h.p.l.c.-u.v. quantitative analysis of 2-(2'-hydroxyphenoxy)pyridine 127

Column : Corasil II ; eluent : cyclohexane, isopropanol 1.5% ; internal standard : 3-hydroxyphenol (resorcinol).

Six solutions containing known amounts of 2-(2'-hydroxyphenoxy)pyridine 127 and 3-hydroxyphenol were prepared. 3-Hydroxyphenol (5.959 g) was taken to volume (100 ml) with a 1:9 methanol-chloroform solution and 5 ml aliquots of such a solution were used (Table 19).

Table 19

Solution	<u>127</u> (mmoles)	3-Hydroxyphenol (mmoles)	Molar ratio <u>127</u> /reference
1	1.75×10^{-2}	27.05×10^{-2}	6.47×10^{-2}
2	4.00×10^{-2}	27.05×10^{-2}	14.78×10^{-2}
3	4.49×10^{-2}	27.05×10^{-2}	16.6×10^{-2}
4	1.09×10^{-2}	27.05×10^{-2}	4.03×10^{-2}
5	4.15×10^{-2}	27.05×10^{-2}	15.34×10^{-2}
6	3.02×10^{-2}	27.05×10^{-2}	11.16×10^{-2}

Each solution was injected twice in different amounts a and b. Each chromatogram was photocopied eight times, the peaks were cut and weighed. The averaged weights are reported in Table 20.

Table 20

Averaged weights of peak areas (mg)

		1	2	3	4	5	6
a	<u>127</u>	9.94 ±8%	18.43 ±4.9%	13.40 ±3.7%	10.13 ±2.9%	34.28 ±4.6%	15.56 ±6.1%
	reference	29.33 ±4%	21.76 ±5%	21.78 ±4.4%	45.04 ±3.3%	44.79 ±3.1%	24.49 ±3.9%
b	<u>127</u>	18.41 ±2%	35.61 ±7.3%	21.83 ±3.4%	4.94 ±10%	28.78 ±6.0%	27.60 ±6.3%
	reference	50.40 ±2.7%	44.46 ±3.1%	44.91 ±3.2%	22.78 ±3.3%	33.80 ±3.4%	42.33 ±1.7%

The averaged peak area ratios are shown in Table 21.

Table 21

Peak area ratios (127/reference)

	1	2	3	4	5	6
a	0.34	0.85	0.62	0.22	0.77	0.64
b	0.36	0.80	0.49	0.22	0.85	0.65
$\frac{a+b}{2}$	0.35 ±12%	0.83 ±11%	0.56 ±8%	0.22 ±13%	0.81 ±9%	0.65 ±10%

Six points could be obtained, the x_i values being the peak area ratios, and the y_i values being the molar ratio 127/reference :

$$x_1 = 0.35 \qquad y_1 = 6.47 \times 10^{-2}$$

$$x_2 = 0.83 \qquad y_2 = 14.78 \times 10^{-2}$$

$$x_3 = 0.56 \qquad y_3 = 16.6 \times 10^{-2}$$

$$x_4 = 0.22 \qquad y_4 = 4.03 \times 10^{-2}$$

$$x_5 = 0.81 \qquad y_5 = 15.34 \times 10^{-2}$$

$$x_6 = 0.65 \qquad y_6 = 11.16 \times 10^{-2}$$

This data represents a good linear correlation ; the curve $y = 0.180x + 0.01$ was obtained on using the least squares method (see preparation 2).

41. Irradiation of 2-phenoxy-pyridine N-oxide boron trifluoride at different concentrations (320 nm). Quantitative considerations using the h.p.l.c.-u.v. technique.

Five different concentration solutions of 2-phenoxy-pyridine N-oxide boron trifluoride were irradiated in dichloromethane at 320 nm (see Table 22).

Table 22

Solution	127-BF ₃ (mmoles)	Concentration	Irradiation time (hours)
1	0.67	0.21x10 ⁻² M	10
2	0.86	0.27x10 ⁻² M	10
3	2.32	0.73x10 ⁻² M	10
4	4.35	1.36x10 ⁻² M	10
5	6.84	2.07x10 ⁻² M	18

The workup procedure was almost identical in each case. The reactor was washed with methanol and the combined methanol-dichloromethane solutions were evaporated. The resulting oils (1, 3, 4 and 5) were stirred in chloroform-methanol (CH₃OH 3%) over alumina. After filtration and evaporation the reaction products were taken to volume with a chloroform-methanol 9:1 solution (1 to 10 ml, 3 to 25 ml, 4 and 5 to 50 ml). Solution 2 was instead passed through a silica column (20 g, eluent chloroform-methanol 3%), evaporated and taken up to 10 ml.

Aliquots (1 ml) were pipetted out from each solution and added to known amounts of 3-hydroxyphenol (Table 23).

Table 23

	3-Hydroxyphenol present in each solution	
Solution 1	11.97 mg	10.87×10^{-2} mmoles
Solution 2	9.58 mg	8.70×10^{-2} mmoles
Solution 3	9.05 mg	8.22×10^{-2} mmoles
Solution 4	9.58 mg	8.70×10^{-2} mmoles
Solution 5	9.39 mg	8.53×10^{-2} mmoles

The five solutions were injected in the h.p.l.c.-u.v. system (Corasil II column ; eluent : cyclohexane, isopropanol 1.5%). One of such chromatograms (from solution 3) is shown in Fig. 66.

Each chromatogram was photocopied five times. The peaks were cut out, weighed and averaged. The results are shown in Table 24.

From the data given in Table 24 the peak area ratios were calculated (Table 25).

Table 24

Averaged weight of peak areas (mg)

	<u>o</u> , <u>127</u>	<u>m</u> , <u>128</u>	<u>p</u> , <u>129</u>	Reference
1	24.31 ±3.9%	1.30 ±7.7%	4.88 ±5.3%	19.40 ±2.0%
2	24.66 ±11.3%	3.24 ±11%	4.96 ±8.0%	19.44 ±2.0%
3	34.96 ±5.0%	2.68 ±56%	7.48 ±53%	18.72 ±2.6%
4	31.78 ±36%	1.16 ±17%	6.52 ±4.6%	20.66 ±1.6%
5	32.64 ±1.8%	1.20 ±13%	5.14 ±5.8%	12.22 ±4.9%

Table 25

Ratios between peak areas

	<u>127/reference</u>	<u>127/129</u>	<u>127/128</u>
1	1.25 ±6%	4.98 ±9%	18.7 ±11%
2	1.27 ±13%	4.97 ±20%	7.61 ±22%
3	1.86 ±8%	4.67 ±10%	13.04 ±11%
4	1.54 ±5%	4.87 ±8%	27.40 ±21%
5	2.67 ±7%	6.35 ±8%	27.20 ±15%

From the curve $y = 0.180x$, five different molar ratios (127/reference) were calculated (Table 26).

Table 26

	x (peak area ratios)	y (molar ratios)
1	1.25	22.5×10^{-2}
2	1.27	22.86×10^{-2}
3	1.86	33.48×10^{-2}
4	1.54	27.72×10^{-2}
5	2.67	48.06×10^{-2}

From the molar ratios (127/reference) obtained in Table 26, and from the amounts of 3-hydroxyphenol present in each solution, the total amounts of 127 were obtained (Table 27).

The total yields of 127 are summarised in Table 28.

These results appear reproducible. The larger yield obtained from solution 1 can be explained by both experimental error and a relatively low amount of 2-phenoxy pyridine N-oxide complex irradiated. The poor yield from solution 2 can be explained by the slightly different workup procedure involved.

Table 27

Total amounts of 127 produced in each irradiation

	Reference (mmoles)	y (molar ratio)	<u>127</u> (mmoles)	Total amount of <u>127</u> (mmoles)
1	10.87×10^{-2}	22.5×10^{-2}	2.45×10^{-2}	24.5×10^{-2}
2	8.70×10^{-2}	22.86×10^{-2}	1.98×10^{-2}	19.8×10^{-2}
3	8.22×10^{-2}	33.48×10^{-2}	2.76×10^{-2}	69.0×10^{-2}
4	8.70×10^{-2}	27.72×10^{-2}	2.41×10^{-2}	24.1×10^{-2}
5	8.53×10^{-2}	48.06×10^{-2}	4.10×10^{-2}	205.0×10^{-2}

Table 28

Total yields of 127

	Concentration of starting material	Irradiation time (hours)	Yield of <u>127</u> (%)
1	$0.21 \times 10^{-2} \text{M}$	10	36%
2	$0.27 \times 10^{-2} \text{M}$	10	23%
3	$0.73 \times 10^{-2} \text{M}$	10	30%
4	$1.36 \times 10^{-2} \text{M}$	10	28%
5	$2.07 \times 10^{-2} \text{M}$	18	30%

42. Irradiation of 2-phenoxypyridine N-oxide boron trifluoride in the presence of benzene (320 nm)

2-Phenoxypyridine N-oxide boron trifluoride (570 mg) in dichloromethane (130 ml) and benzene (80 ml) was irradiated for 6 hours at 320 nm. The reaction mixture was concentrated at the spinning band column (20 mmHg, 30°C). Chloroform-methanol (CH₃OH 5%) was added (35 ml) and the mixture stirred over alumina. After filtration and concentration the solution was taken to volume (25 ml) with chloroform-methanol (CH₃OH 5%). A t.l.c. plate showed the presence of phenol. This spot was absent in all the irradiation experiments in dichloromethane. One aliquot (1 ml) was pipetted out and taken to volume (5 ml) together with some 3-hydroxyphenol reference (33.07 mg, 30.03x10⁻² mmoles). It was not possible to detect the presence of phenol by h.p.l.c.-u.v. technique. Phenol and 2-(2'-hydroxyphenoxy)pyridine had very similar retention times; a shoulder which could well be phenol was distinguished, but no separation could be obtained. Three different amounts of solution were injected and each chromatogram was photocopied seven times; the peaks were cut out, weighed and averaged (Table 29).

The 127/reference and 127/129 peak area ratios were then calculated (Table 30).

From the curve $y = 0.180x$, when $x=0.45$, $y=8.1 \times 10^{-2}$ (molar ratio). As a known amount of 3-hydroxyphenol was present (30.03x10⁻² mmoles), the total yield of 127 was calculated (2.43x10⁻² mmoles in 1 ml; total 0.61 mmoles, 27.2% yield); the yields of 127 do not appear to change substantially in the presence of benzene.

Table 29

Averaged weights of peak areas (mg)

	<u>127</u>	<u>128</u>	Reference
1	9.64 ± 4.0%	2.94 ± 6.8%	22.09 ± 3.6%
2	9.67 ± 6.7%	2.20 ± 9.0%	20.26 ± 6.2%
3	9.60 ± 6.3%	2.44 ± 6.1%	21.95 ± 5.0%

Table 30

Peak area ratios

	<u>127/reference</u>	<u>127/129</u>
1	0.44 ± 8%	3.28 ± 11%
2	0.48 ± 13%	4.40 ± 16%
3	0.44 ± 11%	3.93 ± 12%
Average	0.45	3.87

43. Synthesis of 2-([2',4',6'-²H₃]-phenoxy)pyridine N-oxide 131

a) Preparation of [2,4,6-²H₃]-phenol

Sodium phenate (4 g, 34.4 mmoles) and sodium tert-butoxide (0.5 g) dissolved in deuterium oxide (6 ml) were sealed in a glass tube and heated at 100°C for 9 hours. The solution was acidified with dilute hydrochloric acid and repeatedly extracted with ether (5x20 ml). The ethereal layers were dried over sodium sulphate, filtered and evaporated. Some non-deuterated product was still present (n.m.r.). The reaction was repeated, with identical workup conditions. [2,4,6-²H₃]-Phenol was obtained as a solid (2.95 g, 30.4 mmoles, 88%). The deuteration was checked by n.m.r. :

δ 6.0 (s, 1H, exchangeable with D₂O, phenolic proton),
7.18 (s, 2H, aromatic m-hydrogens). A very small amount of undeuterated phenol (≈ 1%) was still present.

b) Reaction of [2,4,6-²H₃]-phenol with 2-chloropyridine N-oxide 126

Sodium hydride (1.2 g of a 60% mineral oil dispersion, 30 mmoles) was suspended in DMF (25 ml). [2,4,6-²H₃]-Phenol (1 eq.) in DMF (20 ml) was added dropwise at 0°C. When the gas evolution ceased, the reaction was allowed to reach room temperature, and 2-chloropyridine N-oxide 126 (3.1 g, 24 mmoles) in DMF (10 ml) was added. The solution was stirred at 70°C for 2 hours and t.l.c. showed the quantitative formation of a more polar product. Most of the DMF was removed by distillation under reduced pressure (40 mmHg). The solution was treated with chloroform (50 ml) and aqueous sodium hydroxide

(25 ml). The organic phase was separated and the aqueous portions were extracted with chloroform (6x20 ml). The combined organic extracts were dried over sodium sulphate, filtered and evaporated. A solid was obtained (4.1 g) after drying in vacuo. This solid was purified by column chromatography on alumina (250 g; dichloromethane as the eluent ; the polarity was gradually increased with methanol). Crystals (3.7 g, 81% yield), reasonably pure on t.l.c. , were obtained, m.p. 86-88°C. Part was further purified by preparative t.l.c. and colourless crystals (250 mg) were obtained, m.p. 88°C. The structure was checked by i.r., n.m.r. and mass spectroscopic data : $\nu_{\max}$ 3050, 1605, 1575, 1490, 1430, 1300, 1270, 1250, 1190, 1070, 1040, 815, 795 cm^{-1} , δ 6.8-7.6 (m, 5H, aromatic protons), 8.25-8.46 (m, 1H, aromatic proton α to the nitrogen), M/e 190 (M^+ , 0.57%), 189 (M^+-H , 1.95%), 174 (M^+-O , 60.2%), 173 (M^+-OH and ^{13}C isotopic peak of M/e 172, 51.7%), 172 (M^+-OD , 100%), 171 (M^+-HOD , 28.4%).

44. Irradiation of 2-([2',4',6'- $^2\text{H}_3$]-phenoxy)pyridine N-oxide boron trifluoride

The boron trifluoride complex of 2-([2',4',6'- $^2\text{H}_3$]phenoxy)pyridine N-oxide (3.17 mmoles) was dissolved in dichloromethane (300 ml) and irradiated (320 nm) for 10 hours in the Reading reactor. The solution was collected and the reactor rinsed with chloroform-methanol 5:1 (5x20 ml). The combined portions were evaporated and a chloroform-methanol (CH_3OH 4%) solution was added (30 ml). The solution was stirred over alumina (1 g) for 40 minutes, then filtered

and evaporated. Part of the oil obtained was purified by preparative t.l.c. and the usual workup procedure afforded o-hydroxylated product (40 mg) and a mixture of m- and p-hydroxylated products (18.8 mg).

The products were purified by preparative h.p.l.c.-u.v. technique (Porasil T column, 3% isopropanol in cyclohexane as the eluent). The o-phenol was easily purified, whilst the m- and p- compounds could not be completely separated. Approximately 90% enriched mixtures of each isomer were obtained.

The three compounds were submitted to high resolution mass spectrometry :

a) Mass spectral data of the o-hydroxylated product

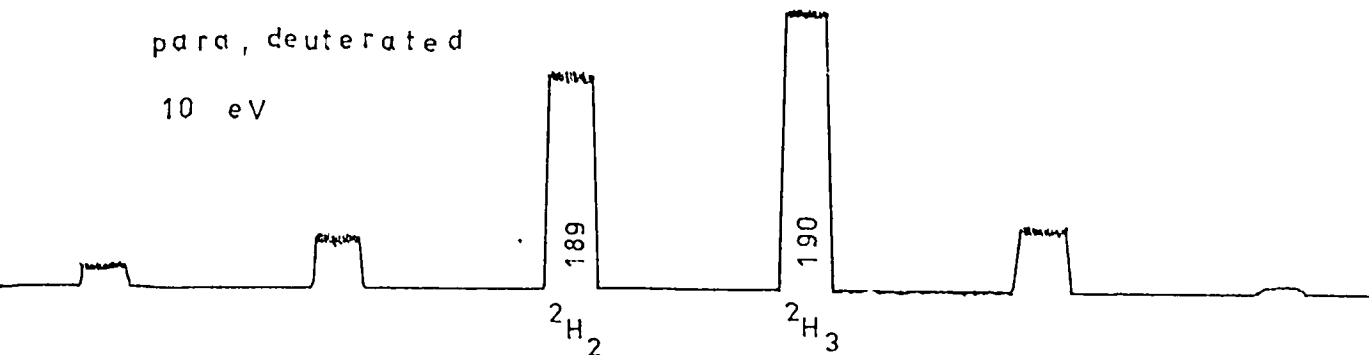
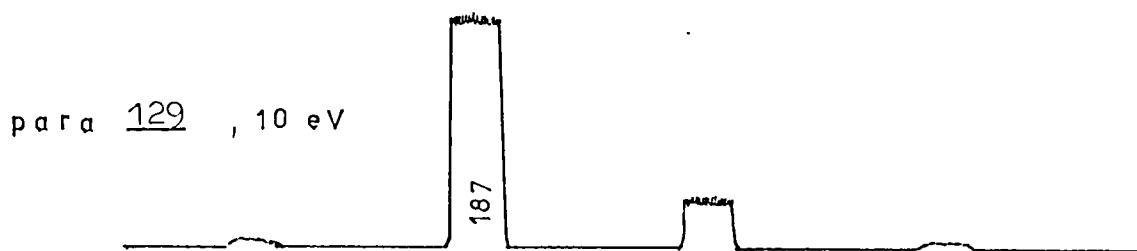
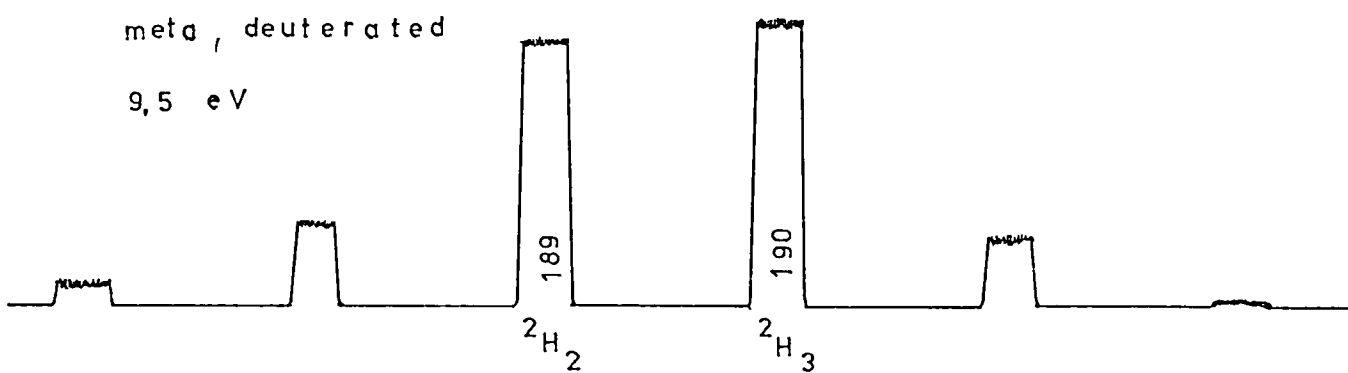
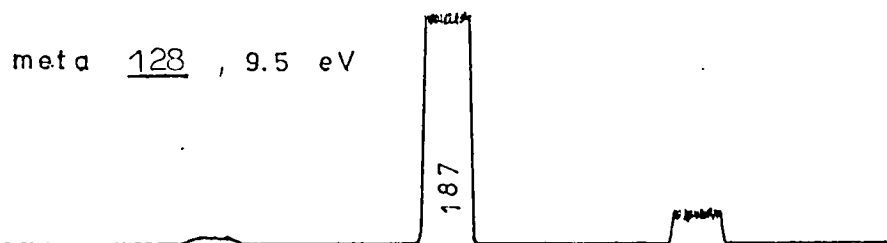
M/e 190 (M^+ of the $^2H_3$ species and ^{13}C isotopic peak of the $^2H_2$ species, 23.96%), 189 (M/e 190-H, and M^+ of the $^2H_2$ species, 34.65%), 173 (M/e 190-OH, and ^{13}C isotopic peak of M/e 172, 56.54%), 172 (M^+ of the $^2H_2$ species-OH, and M^+ of the $^2H_3$ species-OD, 100%).

b) Mass spectral data of the m- and p- hydroxylated products

The m- and p- compounds were checked for deuterium content with the method of Biemann (88). The M^+ region was scanned at low eV (10) to avoid $M-H^+$ and $M-D^+$, and compared to the M^+ region of the unlabelled samples 128 and 129 (Fig. 76). The peaks at M/e 187, 188, 189, 190 and 191 were attributed to the $^2H_0$, $^2H_1$, $^2H_2$, $^2H_3$ and $^2H_4$ species respectively. The m- compound showed a deuterium content as follows : 3% ($^2H_0$), 13% ($^2H_1$), 39% ($^2H_3$) and 5%

Fig. 76

Mass spectra at low ionisation potential of 128 and 129,
and of their corresponding deuterated analogs.



($^2\text{H}_4$). The p- compound showed the following deuterium content : 4% ($^2\text{H}_0$), 9% ($^2\text{H}_1$), 38% ($^2\text{H}_2$), 46% ($^2\text{H}_3$) and 3% ($^2\text{H}_4$). It should however be remembered how both samples are enriched mixtures ($\approx 90\%$), as we were not able to separate the m- from the p- compound by h.p.l.c. on a preparative scale.

45. Preparation of sodium hydroperoxide (80)

Benzhydrol (1935 mg, 10.5 mmoles) and sodium t-butoxide (1010 mg, 10.5 mmoles) were mixed in a round bottom two neck flask (100 ml). Petroleum (40 ml) was added and the mixture was stirred at 0°C . The system was evacuated and O_2 (250 ml, 11 mmoles) was allowed to flush into the system (the gas was contained in a two tap round bottom flask ; dry O_2 was flushed into it and immediately used). A colourless precipitate formed almost immediately. The reaction was allowed to proceed for 45 minutes. Water (5 ml), containing MgSO_4 (10 mg), was added to the reaction mixture, the reaction product quickly dissolving in the aqueous phase. This was separated and the organic layer was repeatedly extracted with water (2x5 ml), containing sodium hydroxide pellets (70 mg). The aqueous phase contained 7.35 mmoles of sodium hydroperoxide (calculated by iodometric titration with 0.1N sodium thiosulphate), 66.8% yield from O_2 .

46. Preparation of [^{18}O]-labelled sodium hydroperoxide

The previous procedure was repeated, $^{18}\text{O}_2$ gas (21.3 atom %) being used (250 ml).

47. Preparation of m-chloroperbenzoic acid (82)

Dioxan (15 ml) was added to the solution of sodium hydroperoxide obtained as described in preparation 45. The reaction mixture was cooled at 0°C and m-chloroperbenzoyl chloride (717 mg, 4.09 mmoles) was added dropwise (3 minutes). The mixture was stirred at 0°C for 15 minutes and then the ice bath was removed and the reaction mixture was allowed to reach room temperature (15 minutes). The pH was alkaline before and after adding the acid chloride. Dichloromethane (20 ml) was added and the mixture was acidified with trifluoroacetic acid in dichloromethane. The organic phase was separated and the aqueous layers were repeatedly extracted with dichloromethane (4x15 ml). Magnesium sulphate and 2,6-di-t-butyl-4-methylphenol (5 mg) were added : the solution was filtered and concentrated under vacuum (20 mmHg pressure) at 25°C . The resulting solution was taken to volume (50 ml). An iodometric titration (2x1 ml aliquot) gave 4.00 mmoles (peracid + peranhydride) (96% yield from the acid chloride, 36% yield from O_2 gas).

48. Preparation of [^{18}O]-labelled m-chloroperbenzoic acid

The previous procedure was employed, the sodium hydroperoxide solution being prepared from $^{18}\text{O}_2$ gas. An iodometric titration gave 4.05 mmoles peracid (and peranhydride): a 98%

yield (from the acid chloride) was obtained. 36% of the $^{18}\text{O}_2$ gas was converted into the peracid.*

49. Preparation of 2-benzylpyridine N-oxide (control experiment)

2-Benzylpyridine (580 mg, 3.43 mmoles) was added to the *m*-chloroperbenzoic acid solution obtained in preparation 47. The mixture was stirred for 30 hours at 25°C and then refluxed for 2 hours. Water (25 ml) was added after cooling. The solution was treated with 1N aqueous sodium hydroxide until alkaline and the organic phase was separated. The aqueous phase was repeatedly extracted with dichloromethane (3x20 ml) and the combined organic extracts were dried over sodium sulphate, filtered and evaporated. A yellow oil (618 mg) was obtained after drying in vacuo. A t.l.c. confirmed the formation of 2-benzylpyridine N-oxide. An n.m.r. spectrum indicated a 69% conversion : δ 4.07 (s, benzylic protons of 2-benzylpyridine : 3.2 cm), 4.20 (s, benzylic protons of 2-benzylpyridine N-oxide : 7.2 cm).

50. Preparation of ^{18}O -N-labelled 2-phenoxy pyridine N-oxide
130

2-Phenoxy pyridine (598 mg, 3.49 mmoles) was added to the labelled peracid solution obtained in preparation 48. An identical procedure to the one adopted in the control preparation 49 was carried out. A yellow oil (500 mg) was obtained, which appeared to be a mixture of 2-phenoxy pyridine and 2-phenoxy pyridine N-oxide (t.l.c., n.m.r.). The crude

* An amount of peranhydride was present.

reaction mixture was purified by preparative t.l.c.. The more polar band afforded some colourless crystals (350 mg, 17% yield from O_2), m.p. $88^\circ C$. The m.p. of 2-phenoxy pyridine N-oxide reference is identical. The labelling was checked with high resolution mass spectroscopy: $[^{18}O]$ -N-oxide : M/e 189 (M^+ , 0.09%), 188 (M^+-H , 0.24%) ; $[^{16}O]$ -N-oxide : M/e 187 (M^+ , 1.33%), 186 (M^+-H , 1.97%). From the molecular ion peak intensities, the amount of ^{18}O labelled compound is 6.3% ; from the peaks due to loss of hydrogen (which are more intense), the amount of ^{18}O labelled compound appears to be 10.8%. Quantitative considerations with low intensity peaks are anyhow not indicative.

51. Irradiation of the boron trifluoride complexes of 2-($[2',4',6' - ^2H_3]$ phenoxy)pyridine N-oxide and 2-phenoxy-pyridine $[^{18}O]$ -labelled N-oxide (320 nm)

The boron trifluoride complexes of 2-($[2',4',6' - ^2H_3]$ phenoxy)pyridine N-oxide (190.8 mg) and of the $[^{18}O]$ -labelled 2-phenoxy pyridine N-oxide (187.7 mg) were prepared as in experiment 6.

The compounds were irradiated in dichloromethane (200 ml) for 8 hours (320 nm). The reaction mixture was separated and the reactor rinsed with chloroform-methanol 1:1 (6x30 ml). The combined portions were evaporated and an oil was obtained. A chloroform -methanol 9:1 solution was added (25 ml) and the solution was stirred over alumina (2 g) for 2 hours. After filtration and evaporation a brown oil was obtained, which gave four products on t.l.c. Part

of the mixture was purified by preparative t.l.c. and four components were obtained : I, oil, 2-phenoxy pyridine (23.4 mg) ; II, solid, o-hydroxylated product (63.2 mg) ; III, solid, m- and p-hydroxylated products (25.3 mg) ; IV, solid, starting material (31.9 mg).

Compounds I and IV were identified by comparison with references. Compounds II and III were purified by h.p.l.c.-u.v. technique (Porasil T column). Pure o-hydroxylated compound was obtained ; approximately 90% enriched mixtures of the m- and p-phenols were obtained. The o-compound was submitted to high resolution mass spectrometry and no peak at M/e 192 could be detected (such a peak should be due to the species with ^{18}O and $^2\text{H}_3$).

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