TRENT POLYTECHNIC

A STUDY OF THE OXIDATION OF SOME PHENOLIC 1,2,3,4-TETRAHYDROISOQUINOLINE-1-CARBOXYLIC ACIDS

being a thesis submitted to

The Council for National Academic Awards

for the degree of

DOCTOR OF PHILOSOPHY

by

Edward John Tinley B.Sc.

October 1977

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

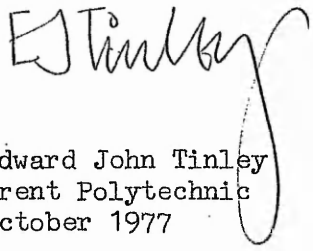
PREFACE

The work described in this thesis was carried out by the author in the Department of Physical Sciences, Trent Polytechnic, Nottingham, between October 1974 and October 1977. A few compounds were obtained from other workers both within the department and from external sources, acknowledgement being made in the text where appropriate.

The author wishes to thank Dr. I.G.C. Coutts for his excellent supervision and Drs. J.M. Barker and P.R. Huddleston for helpful advice. Thanks are also due to Mr. M.L. Wood, Mr. B. Peutrell and Mr. M. Healey for spectral determinations and to Mrs. S. Roller for typing this thesis.

The author is also endebted to the Science Research Council for the provision of a research studentship between the above dates.

Part of the work described in Chapter Two was presented at the Sixth Natural Products Symposium, University of the West Indies, Mona, Jamaica, January 1976.



Edward John Tinley
Trent Polytechnic
October 1977

SUMMARY

Following the publication of a report on the anodic oxidation of 6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acids a range of such compounds with differing alkyl substituents at the C-1 position was synthesised and incubated with enzymes known to oxidise phenolic molecules. It was found that oxidative decarboxylation to the corresponding 3,4-dihydroisoquinoline occurred, the reactions being followed spectrophotometrically, and although a competing reaction was also observed an estimate of the enzyme kinetics (Km and V) was made. This reaction is additional evidence in favour of the Hahn postulate implicating isoquinoline-1-carboxylic acids in the biogenesis of isoquinoline alkaloids, and also illustrates the similarities between enzymatic and electrochemical oxidations.

As a consequence of the reported oxidative decarboxylation of phenolic N-protected acids to quinonoidal species, two N-trifluoroacetyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acids, having nucleophilic benzyl substituents were synthesised and oxidised by electrochemical, enzymatic and chemical means, in order to study their possible intramolecular coupling to aporphine and cularine alkaloids. Synthesis of the target alkaloids by conventional methods was also undertaken. Similarly the possibility of intermolecular coupling of an external nucleophile to the oxidised form of N-trifluoroacetyl-1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid was investigated, both electrochemically and enzymically.

An unsuccessful synthesis of β,β-dimethyl-3,4,5-trimethoxyphenylpyruvic acid was undertaken in an attempt to prepare the appropriately substituted α,α-dimethyl isoquinoline-1-carboxylic acid.

Reaction of 1-(3′,4′,5′-trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid with trifluoroacetic anhydride in pyridine gave rise to an anomalous product, which was identified as a fluorine containing dihydroprotoberberine.

The enzymatic oxidation of corypalline was investigated using fungal laccase and horseradish peroxidase; both enzymes were found to be capable of effecting phenol oxidative coupling to give the carbon-carbon linked dimer.

TABLE OF CONTENTS

ABBREVIATIONS

DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMF	N,N-dimethylformamide
DMSO	dimethylsulphoxide
Et	ethyl
Me	methyl
n.m.r.	nuclear magnetic resonance spectroscopy
Ph	phenyl
TFA	trifluoroacetic acid
TFAA	trifluoroacetic anhydride
THF	tetrahydrofuran
t.l.c.	thin-layer chromatography
u.v.	ultra-violet spectrophotometry

Novel compounds

Compounds, the names of which are underlined in both text and experimental sections, have not (to the best of the author's knowledge) been described in the literature.

CHAPTER ONE

INTRODUCTION

In a recent paper[1], Bobbitt reported that 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acids 1 possessing either a 6- or 7-hydroxy function on the isoquinoline nucleus can be decarboxylated by anodic oxidation at low potentials, to produce the corresponding 3,4-dihydroisoquinolines 2 in high yields. Such findings lend support to the proposal made by Hahn[2] that isoquinoline-1-carboxylic acids are involved in the biosynthesis of isoquinoline alkaloids. Based upon the experimental conditions, it was considered likely that certain electrochemical oxidations give results similar to those occurring *in vivo*. It was further postulated[1] that such a decarboxylation induces a "push-pull" effect across the phenolic isoquinoline system, generating in the case of the 6-hydroxy acids, intermediate quinone-type compounds 3, which can subsequently rearomatise by involving the nitrogen lone pair electrons to give the 3,4-dihydro products. The possibility would therefore seem to exist that if these lone pair electrons are prevented from taking part in the aromatisation reaction, e.g. by N-acylation, the generated quinone can either be isolated, or can react with some nucleophilic species. Indeed Bobbitt, isolated a quinonoidal compound in low yield from the electrochemical oxidation of an N-acetylated isoquinoline-1-carboxylic acid, and suggested that such quinones may be involved in the formation of isoquinoline alkaloids by either intra-or intermolecular nucleophilic addition, without the necessity of further oxidation. Such a process would be an example of what Hamilton[3] has termed non-oxidative coupling (NOC).

In view of the importance of these proposals, a study was undertaken to investigate:-

a) the relationship between anode- and enzyme-induced oxidative decarboxylations of phenolic isoquinoline-1-carboxylic acids;

b) the possible synthesis of intra- and intermolecularly coupled products from the decarboxylation of N-acylated acids by electrochemical, chemical and enzymic oxidations.

The remainder of this introduction will discuss the role of phenol oxidative coupling and isoquinoline-1-carboxylic acids in the context of the biosynthesis of isoquinoline alkaloids.

1.1 Phenol Oxidative Coupling

1.1.1 Mechanistic Considerations

Of the many ring systems that are present in naturally occurring systems, phenols are one of the most easily oxidised. The products from such oxidations are often complex mixtures of dimers, polymers and quinonoidal compounds, depending upon the type of oxidant and the reaction conditions used. The importance of this oxidative coupling process has long been recognised and, as a consequence, many biosynthetic pathways involving such reactions have been proposed, along with attempts to produce this natural process *in vitro*.[4-14]

Some fifty years ago, Robinson[15] related the phenol oxidative coupling process to the biosynthesis of morphine alkaloids from phenolic 1-benzylisoquinolines. Similarly, Pummerer[16] proposed that a process of this type occurred in the chemical oxidation of 4-cresol to give dimeric products. However, until the influential paper by Barton and Cohen[4], the significance of phenol coupling in natural product chemistry

was not widely appreciated. In this publication, the coupling process was rationalised in terms of phenoxy radicals, in which the distribution of the unpaired electron can be represented by the canonical forms 4a-d. The application of ortho, para coupling rules enables only carbon-carbon or carbon-oxygen-carbon coupled products 5-14, linked at the ortho or para positions to be formed.

In general, phenol coupling has been assumed to operate by a radical dimerisation mechanism probably because most of the early investigations involved known one-electron oxidants, e.g. alkaline ferricyanide, ferric chloride, silver oxide and manganese dioxide. Nevertheless, in spite of what appeared to be an abundance of evidence in favour of a radical dimerisation mechanism, Barton[17] suggested that alternative mechanisms may also be involved under certain conditions and should therefore be considered as possible pathways. The following mechanisms were proposed:-

1 $ArO^{\bullet} + ArO^{\bullet} \longrightarrow$ Dimer

2 $ArOH^{+\bullet} + ArO^{\bullet} \longrightarrow$ Dimer + $H^{\oplus}$

3 $ArOH^{+\bullet} + ArOH^{+\bullet} \longrightarrow$ Dimer + $2H^{\oplus}$

4 $ArOH + ArO^{\oplus} \longrightarrow$ Dimer + $H^{\oplus}$

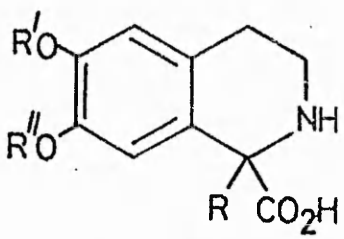
5 $ArO^{\ominus} + ArO^{\oplus} \longrightarrow$ Dimer

6 $ArO^{\bullet} + ArOH \longrightarrow$ Dimer + $H^{\bullet}$

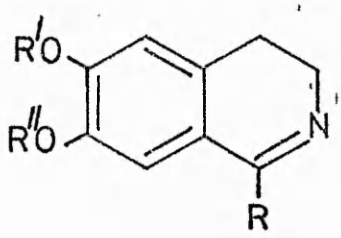
7 $ArO^{\bullet} + ArO^{\ominus} + H^{\oplus} \longrightarrow$ Dimer + $H^{\bullet}$

8 $ArO^{\bullet} + ArO^{\oplus} + e^{\ominus} \longrightarrow$ Dimer.

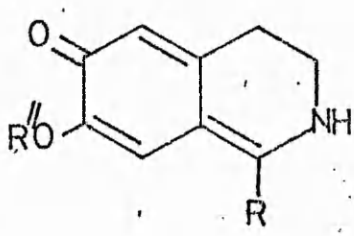
Reactions 1-3 correspond to simple coupling reactions of phenoxy radicals in various stages of protonation, with reaction 1 being the most commonly occurring. Reactions 6-8 are phenoxy radical substitutions, still involving a two-electron transfer process as with coupling reactions. Reactions of type 6 were considered improbable.[10] Reaction 7 was not dismissed outright in view of the evidence presented for its



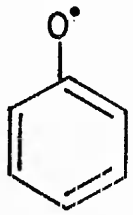
1



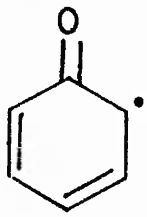
2



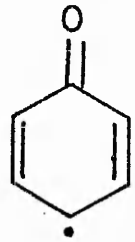
3



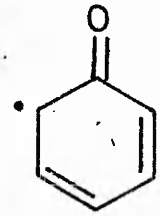
4a



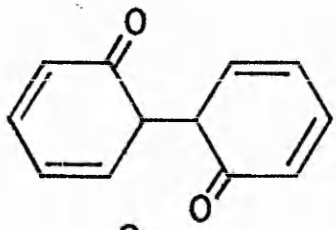
4b

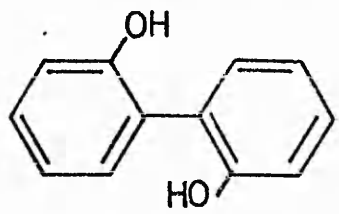


4c

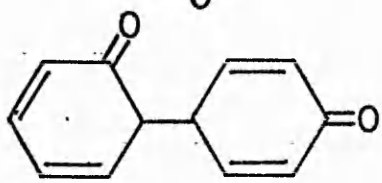


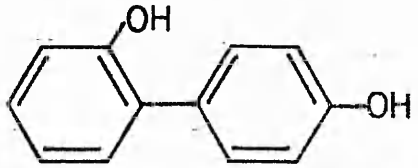
4d

5 

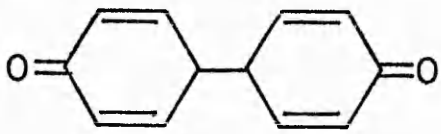


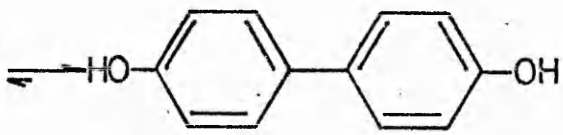
10

6 

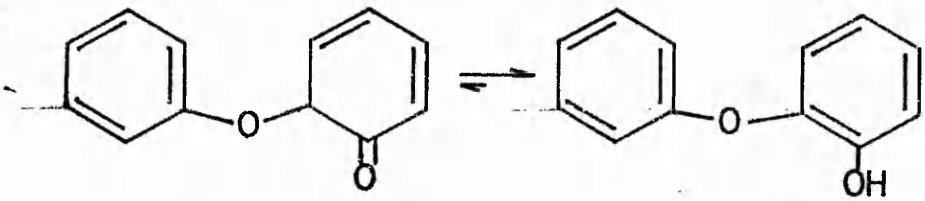


11

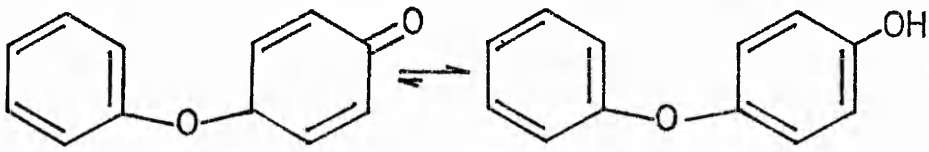
7 



12

8 

13

9 

14

occurrence,[18] whereas reaction 8 was considered extremely unlikely, involving the collision of "two improbable particles". Phenoxy cations are involved in both reaction 4 and 5 and it is towards the identification of such a species that much work has been recently focused. In addition, the species involved in mechanism 3, i.e. a phenoxy cation radical, has received increasing attention with the dramatic growth of electrochemical methods in organic synthesis.

A recent review[3] divides the more common external oxidations into two mechanistic groups which are further subdivided into several general types.

Type 1:- mechanisms involving free radical intermediates;

a) direct coupling of two phenoxy radicals,
b) homolytic aromatic substitution,
c) heterolytic coupling preceeded by two successive one-electron oxidations.

Type 2;- mechanisms which do not involve radicals;

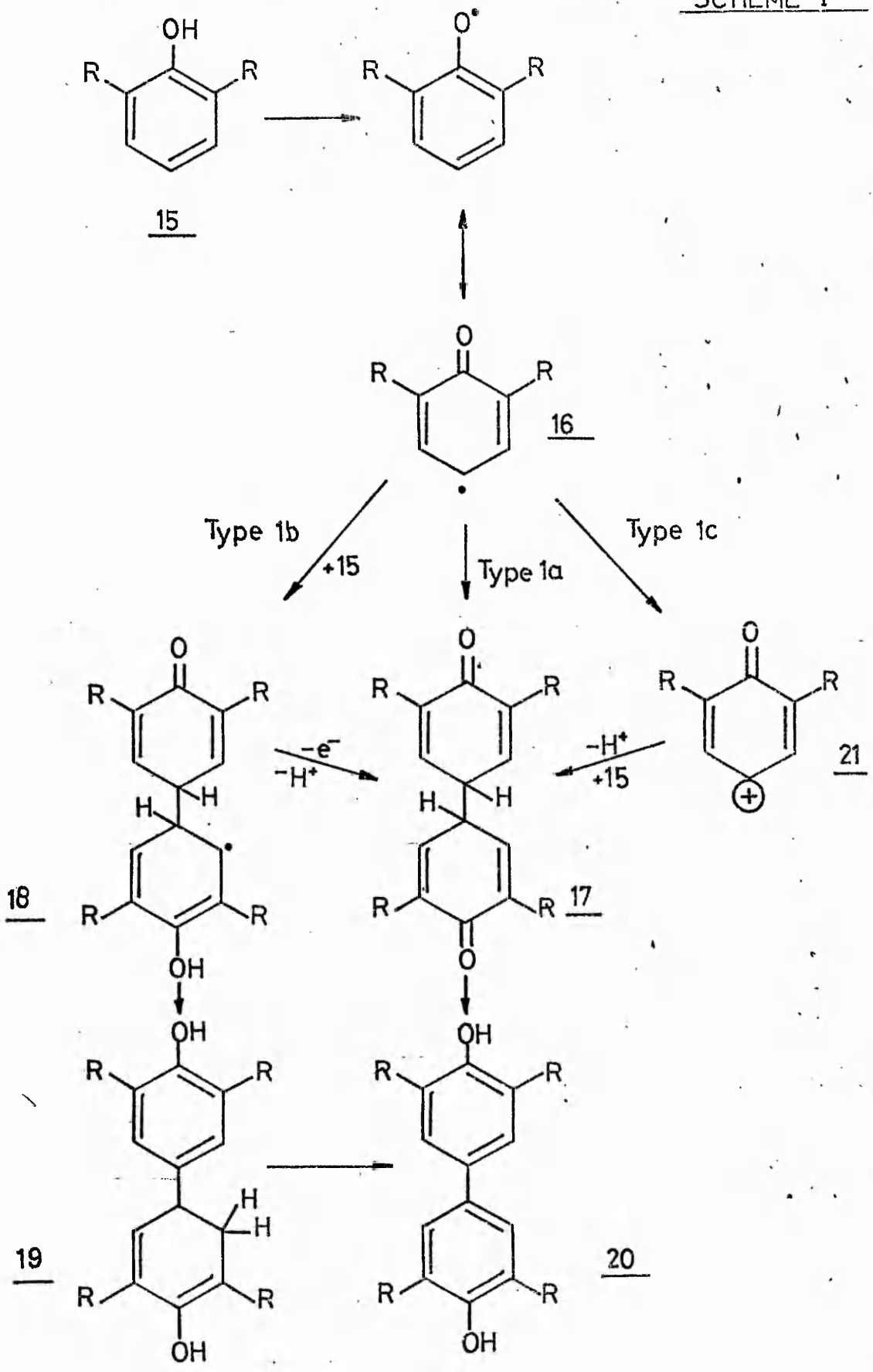
a) heterolytic coupling preceeded by a single two-electron transfer,
b) concerted coupling and electron transfer.

The following sections discuss in some detail the evidence for and against the involvement of radical coupling, homolytic substitution, and phenoxy cations in phenol oxidative coupling reactions.

1.1.2 Mechanisms Involving Initial Phenoxy Radical Formation

Para coupling of the phenoxy radical from 2,6-dialkylphenol 15 illustrates the above three mechanisms involving radical intermediates (Scheme I). The same final product 20 can arise from the radical 16 by any of the pathways illustrated. The dicyclohexadienone 17 is formed by homolytic coupling (Type 1a), followed by rapid tautomerisation in protic media to give 20. Homolytic substitution (Type 1b)

SCHEME I



into another phenol molecule generates the dimeric radical 18 which either gives 17 by loss of a proton and an electron, or it may disproportionate to 17 and the dihydro compound 19. Compounds of type 19 are analogous to products observed in free radical aromatic substitutions,[19] but such species have never been identified in phenol coupling reactions, for which the most probable explanation is the greater stabilisation afforded by rearomatisation. Absence of any evidence for such species does not necessarily mean that dihydro intermediates are not involved. The final free radical intermediate pathway (Type 1c) may occur after further oxidation of the phenoxy radical 16, generating a phenoxy cation 21 capable of initiating electrophilic substitution with a phenol molecule to produce 17.

It has been suggested[20] that carbon-carbon coupled products arise as a result of electrophilic substitution by phenoxy cations (such species probably existing in the mesomeric carbonium ion form), and are formed by the acid-induced disproportionation of the corresponding radical:

$$2ArO\cdot + H_3O^{\oplus} \longrightarrow ArO^{\oplus} + ArOH + H_2O$$

whereas carbon-oxygen-carbon products are more likely to involve phenoxy radical coupling since more of the unpaired electron density resides at the oxygen atom. A recent detailed study[21] of the oxidation of 4-cresol to its carbon-carbon dimer has shown that the reaction pathway is not dependent on either redox potential or pH, casting some doubt on the earlier proposal of an ionic mechanism being involved in the formation of carbon-carbon coupled products.

Evidence for phenoxy radical formation

There would appear to be indisputable evidence that the first step of the majority of phenol oxidations involves the removal of one electron

by an external oxidant to give a phenoxy radical. Due to the reactive nature of such species it has, with a few exceptions, only been possible to identify phenoxy radicals by physical means, utilising the properties of the unpaired electron. Esr spectroscopy has proved the most informative method,[22,23] and by this technique it has been possible to assign spin densities to the phenoxy radicals under investigation. Such radicals are deeply coloured in contrast to the parent phenols which are usually colourless, and it has thus been possible to study such species from their electronic spectra.[24] Indirect evidence for the formation of phenoxy radicals is also obtained from redox titration curves and from polarograms of phenols, with a single electron transfer being observed in both cases. Stable phenoxy radicals are known,[25,26] and in most cases are substituted in the 2,4 and 6 positions by groups able to exert steric interference or capable of increasing the delocalisation of the unpaired electron. Such radicals can be isolated and their physical properties easily studied. The existence of radicals derived from phenols has therefore been well established by physical and chemical techniques.

Phenoxy radical dimerisation

There can also be little doubt that in many phenol oxidations the direct radical pairing mechanism (Type 1a) is responsible for the dimeric products observed. Evidence from flash photolysis experiments[27,28] has demonstrated the disappearance of phenoxy radicals by a process following second-order kinetics, attributable to the rapid and irreversible rate determining radical dimerisation process. A recent study[29] has suggested that radicals from highly substituted phenols disproportionate to quinone methides and the parent phenols rather than

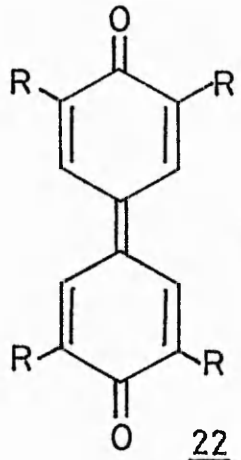
dimerising, in agreement with the earlier observations of Cook and his co-workers.[30,31] Results from the mechanistic studies on relatively simple phenolic systems by Waters[32] and Littler,[33] were consistent with the dimerisation process having occurred. Additional evidence for this pairing mechanism comes from potential cross-coupled product experiments. Ferricyanide oxidation of a mixture of 2,6-dimethyl- and 2,6-dimethoxyphenols afforded only the symmetrical tetramethyl- and tetramethoxydiphenoquinones 22 and 23. Similarly the ferricyanide oxidation of 4-cresol in the presence of excess 1,2-dimethoxybenzene produced no ccupled product in which the dimethoxy compound had been incorporated.[10] Oxidation of the monoether 24 with ferricyanide gave only dimeric products, whereas the diphenol 25 produced[10] the intramolecularly linked bis-spirodienone 26.

Homolytic substitution

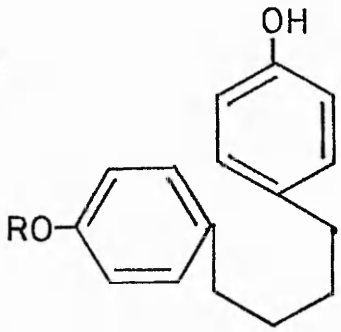
In contrast to the wealth of information implicating the radical dimerisation process, only one piece of evidence has been reported claiming involvement of a homolytic substitution mechanism.[34] Ferricyanide oxidation of 27 produced predominantly the substituted xanthone 28 according to the mechanism proposed in Scheme II.

Heterolytic coupling

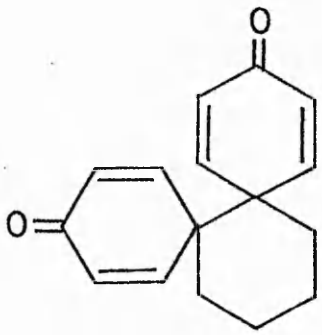
Further oxidation of phenoxy radicals to the corresponding cations requires additional energy. Such a process is therefore the least favourable of the radical initiated mechanisms. However evidence is available for the implication of this species in certain types of reaction, i.e. those in strongly acidic media and in anodic oxidations. The formation of phenoxy cations or their equivalent has been shown to occur in several electrochemical investigations.[35-39] Ronlan and



22 R=Me
23 R=MeO

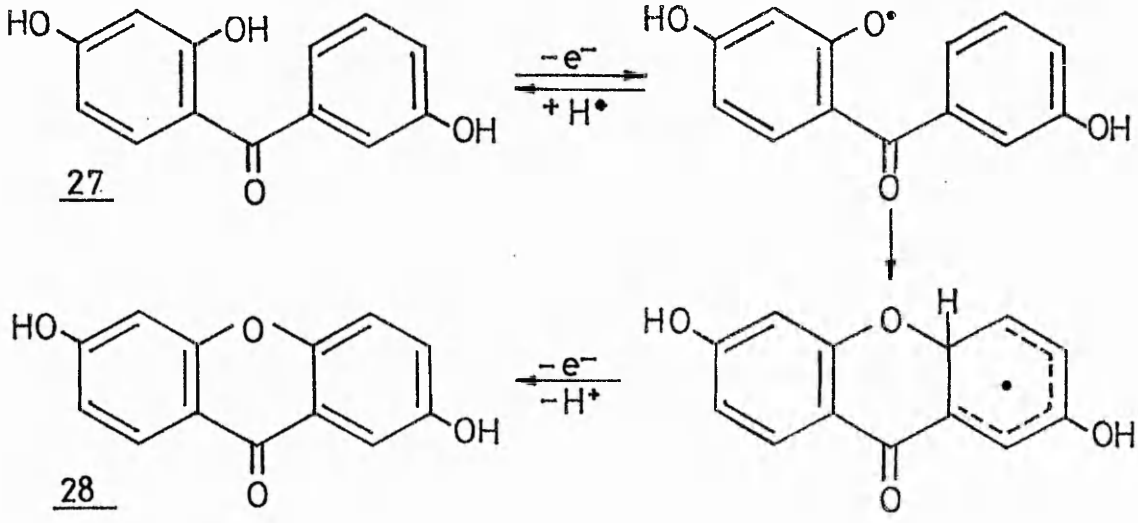


24 R=PhCH$_2$
25 R=H



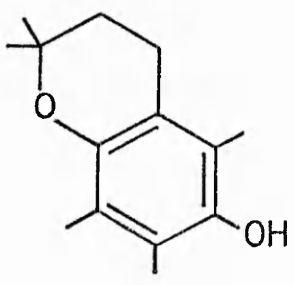
26

SCHEME II

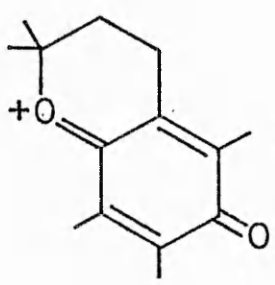


27

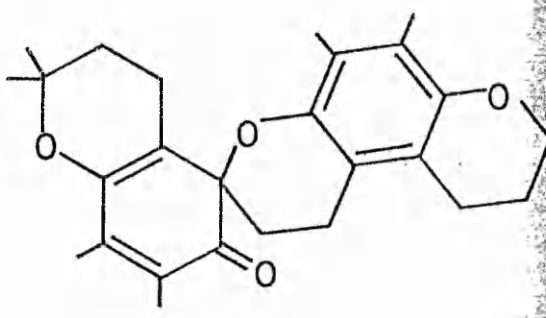
28



29



30



31

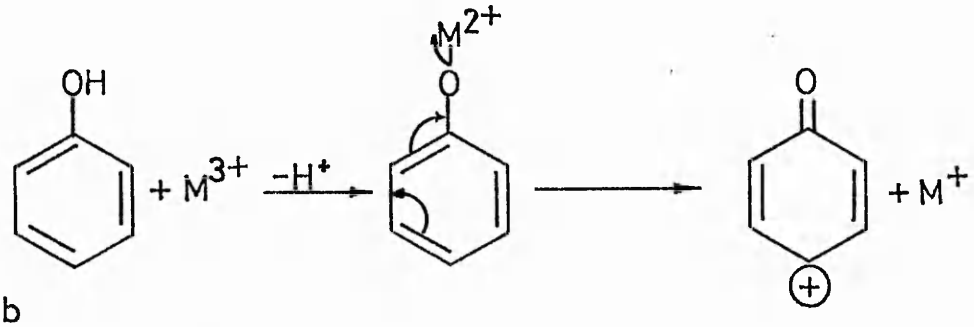
Parker[40,41] have studied the anodic oxidation of a series of monophenolic diphenylpropanes to the appropriate spirodienones. Their findings suggest that a phenoxy cation is an intermediate in the oxidation of several of the compounds investigated. Furthermore, the formation of these ionic intermediates was considered to take place either directly from the phenol by a direct two-electron loss, or from a phenol ether cation radical by an intramolecular charge transfer process. Electrophilic attack by the resultant cation then takes place upon the unoxidised part of the molecule. The same authors demonstrated[42] that the yield of products derived by a phenoxy cation increased when an electrode capable of adsorbing the initially formed phenoxy radicals was used, thus preventing radical dimerisation and enhancing further oxidation to cationic intermediates. Similar oxidation[43] of α-tocopherol 29 resulted in a cation 30 of remarkable stability and the intermediacy of this same ionic species was considered[44] likely in the ferricyanide oxidation of 29 to its dimer 31. Hewitt,[45] although not actually demonstrating the involvement of a phenoxy cation in the coupling process of two phenolic nuclei, considered that for some substrate-reagent combinations, an ionic mechanism more adequately explained the findings. Trapping of cations of this type has also been achieved. The tetrafluoroborate and the hexachloroantimonate salts were isolated[46] as a result of the disproportionation of phenoxy radicals in the corresponding anhydrous strong acid. Similarly, such cationic species have been captured by nucleophiles present in the reaction media.[39,47]

Cation radical mechanisms

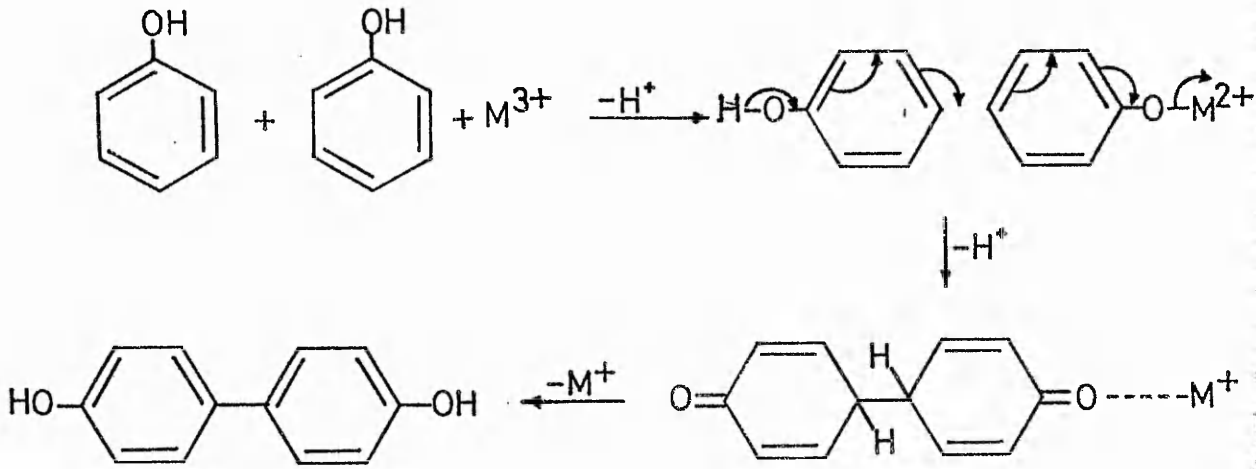
Cation radicals have been implicated in many chemical reactions and

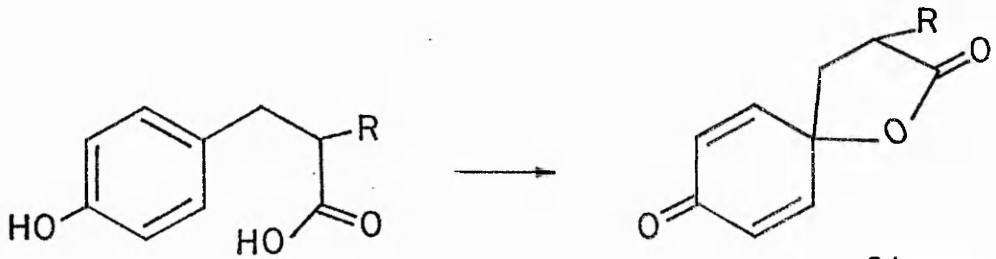
SCHEME III

type 2a



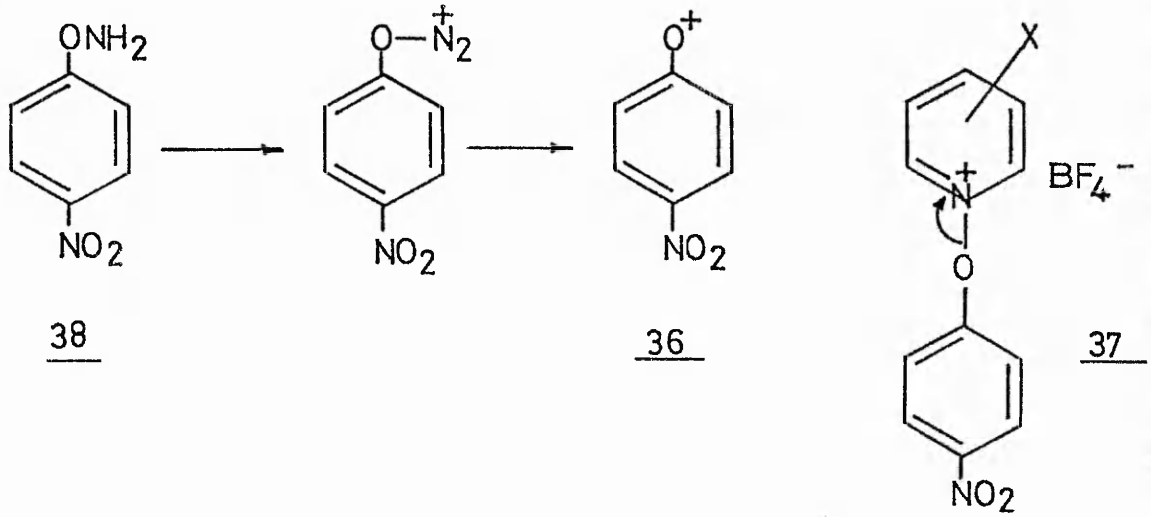
type 2b





32 R=H
33 R=NHCOCH3

34 R=H
35 R=NHCOCH3



38

36

37

have been recently reviewed.[48,49] Electrochemical oxidation of phenol ethers to the corresponding intra or intermolecularly coupled products has been carried out by several groups of workers,[50-53] who have proposed mechanisms generally involving either cation radicals or diradicals. More recently, a mechanism involving an intramolecular coupling reaction between a phenol ether cation radical and a phenoxy radical derived from a diarylpropane was considered to occur under certain conditions.[41] A similar mechanism involving cyclisation of an ether cation radical with the unoxidised phenolic part of the molecule was thought unlikely when the nature of the products was considered.

1.1.3 Mechanisms Not Involving Phenoxy Radicals

The mechanistic processes involved in non-radical coupling are illustrated in Scheme III. Although the external oxidant is shown as a tripositive species, other metals in different oxidation states or organic compounds can also be involved. The initial oxidant-phenol complex either decomposes to give a formally charged phenoxy cation (Type 2a) with its subsequent electrophilic type reactions, as previously discussed, or as seems more likely on energetic grounds, it can break down by a concerted two-electron transfer pathway (Type 2b). Intramolecular coupling of phloretic acid 32 and of N-carbomethoxy-tyrosine 33 to the respective spirolactones 34 and 35, were both considered to occur by a non-radical process.[54,55] Several metals, e.g. Pb (IV), V (IV), V (V), Tl (III) and Mn (III), are known to complex with the hydroxy function of a phenol; the complex may decompose to give a phenoxy cation or to induce a concerted electron transfer process. The earlier findings from lead tetraacetate oxidations of

phenols have been reviewed.[56] Results from a study[57] of the lead tetraacetate oxidation of 2,4,6-tri-tert-butylphenol lend strong support to the involvement of a metal-phenol compound, which heterolytically breaks down either by a Type 2a or a Type 2b route, with concurrent phenol oxidation.

The mechanism for the oxidation of phenols by vanadium compounds is seemingly more complex than for lead tetraacetate, and as yet it is not absolutely clear whether vanadium (IV) and vanadium (V) act as one- or two-electron oxidising agents, although it has been established that a phenol-metal complex is involved.[58] Carrick and his collaborators[59] have suggested that phenol coupling occurs by rearrangement of electrons within a complex containing at least two phenol residues and at least one vanadium atom. In a recent paper,[60] Schwartz proposed that coupling of diphenolic 1,3-diarylpropanes with vanadium oxychloride takes place by diradical dimerisation, in contrast to monophenolic compounds of this same system, which he considered to involve two successive one-electron oxidations with coupling occurring by a cation radical dimerisation process.

Phenoxy cations have been postulated[61] to arise from the use of thallium trifluoroacetate, although other workers[41] implicate a concerted (Type 2b) process. It has also been suggested[62] that thallium (III) salts operate by ipso-thallation of the aromatic ring, followed by nucleophilic displacement of the thallium.

Quinones are known to abstract hydride ions from hydroaromatic compounds, resulting in cationic intermediates. By analogy, it should be possible to generate phenoxy cations directly by a two-electron

process. Thus the cyclisation of 2-hydroxy-3'-methoxybenzophenones to the corresponding xanthones by DDQ was considered[63] to proceed via a phenoxy cation intermediate, i.e. a Type 2a mechanism. Similarly the oxidation of 6-hydroxytetralin to 6-hydroxy-1-tetralone was interpreted in this way.[64] It has also been suggested[65] that DDQ oxidises phenols to the corresponding quinone methide-type intermediates. Both pterocarpan and chromene compounds were considered to be formed through the intermediacy of such compounds, with subsequent intramolecular, non-oxidative coupling reactions.

In a recent significant paper,[66] Abramovitch proposes the intermediacy of the free phenoxy cation 36 in several reactions. This cation was considered to be produced from the thermolysis of various N-aryloxypyridinium salts 37 and from diazotisation of 4-nitrophenoxyamine 38. Subsequent attack of 36 on solvent molecules showed no difference in the isomer ratios of the products, irrespective of the source of 36 and the nature of the pyridinium ring substituent, arguing against a concerted mechanism.

1.1.4 Non-Oxidative Coupling Reactions (NOC)

Strictly speaking, reactions characterised as non-oxidative coupling are further examples of phenol coupling by non-radical-means, and such a process is of particular interest in the biosynthesis of natural products. As mentioned earlier (see page 1) the formation of a quinone type structure leads by a Michael type addition either to intra- or intermolecularly coupled products, in which the quinone structure acts as an internal oxidant, e.g. the oxidation of laudanosoline methiodide by ferric chloride (Scheme IV). Although ferric

chloride is illustrated as the external oxidant required to produce the quinone intermediate, the possibility exists that a suitable enzyme could equally well function as the oxidant. A mechanism therefore seems possible in which coupling of aryl units would be achieved without the involvement of either formally charged or odd electron intermediates, or unnatural oxidants. Furthermore, the only requirement for an NOC process is that one of the components is capable of being reduced by a reasonable mechanism, and quinones are not essential for such coupling. A hypothetical example is the coupling of benzyl alcohol and phenol to give the biphenyl 39.

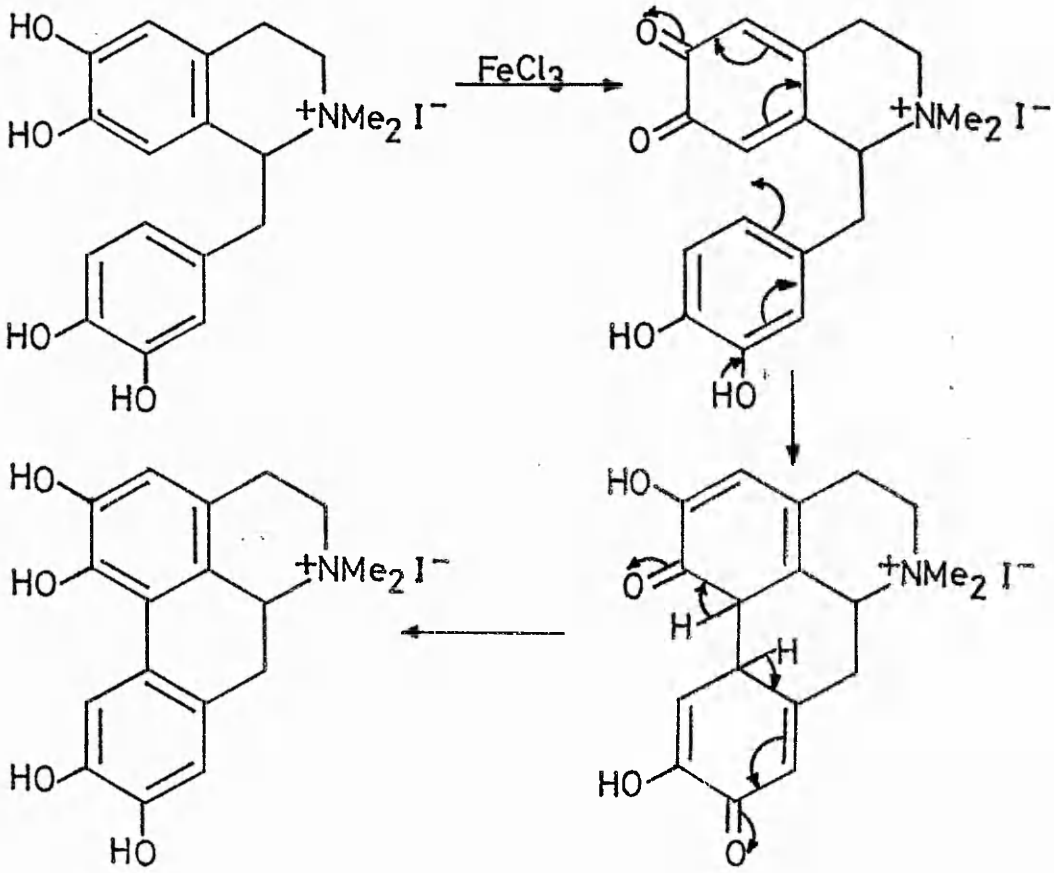
1.2 Phenol Oxidative Coupling In The Biogenesis Of Isoquinoline Alkaloids

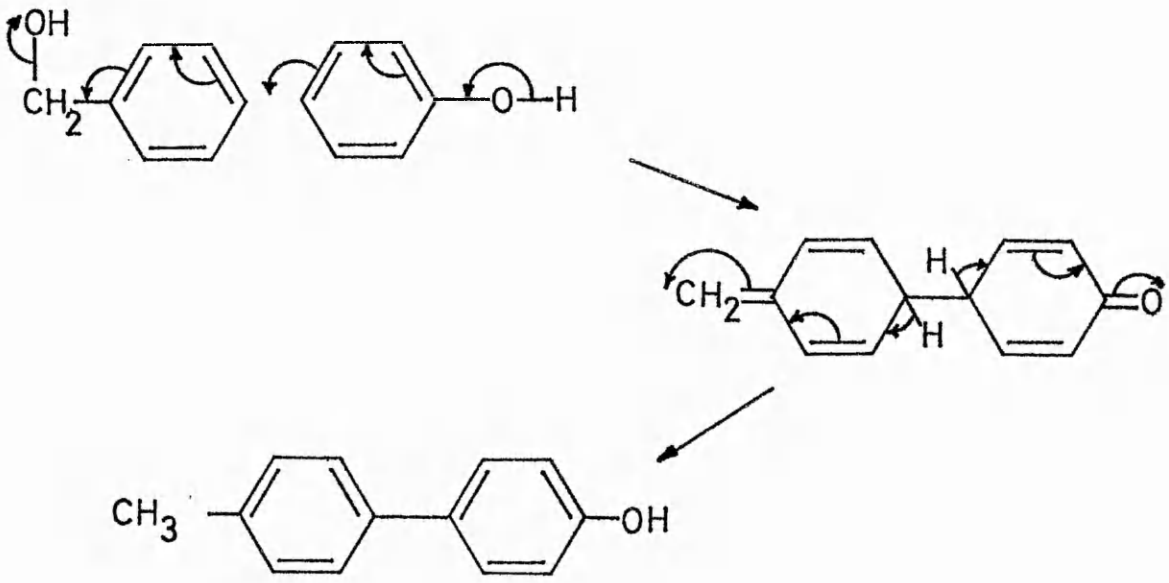
Phenol oxidative coupling reactions either of the inter- or intramolecular typ have been postulated in the biogenesis of many naturally occurring compounds. Since the work involved in this thesis is primarily concerned with intramolecular coupling reactions in isoquinoline molecules, it is intended to restrict the following discussion to topics related to this subject.

The isoquinoline alkaloids are a group of secondary metabolites which have been extensively studied by many groups, both because of their pharmaceutical utility and because of the intrinsic interest of the chemistry of such complex structures possessed by many of the members of this group. Increasing attention has been paid in recent years to the biogenesis of the alkaloids, i.e. to how they arise in plant forms from simple or complex precursors by natural processes.

As early as 1917, Robinson[67] suggested that 1-benzyl-1,2,3,4-tetrahydroisoquinoline compounds could be synthesised in the laboratory

SCHEME IV





39

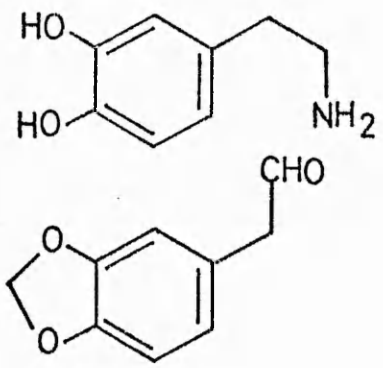
under so called "physiological" conditions, i.e. with reactant concentration, acidity and temperature similar to those found in plants. This hypothesis was later shown to be correct by Schöpf,[68,69] who synthesised the 1-benzylisoquinoline 40 by condensing dopamine 41 with the aldehyde 42 at 25° and pH6. Robinson[15] also conceived the idea that phenol coupling of appropriately substituted 1-benzylisoquinoline compounds could lead to alkaloids of the morphine group. This theory has not only been shown to be correct but has been expanded upon, until it is now generally accepted that phenol oxidative coupling is responsible for many of the alkaloids, and particularly the isoquinoline alkaloids found naturally, e.g. aporphines, homoaporphines, cularines, bisbenzylisoquinolines and the erythrina alkaloids, in addition to the morphine family. A recent review[70] of the biosynthesis of alkaloids derived from 1-benzylisoquinolines supplements the earlier excellent review by Battersby.[71]

1.2.1 The Aporphinoids

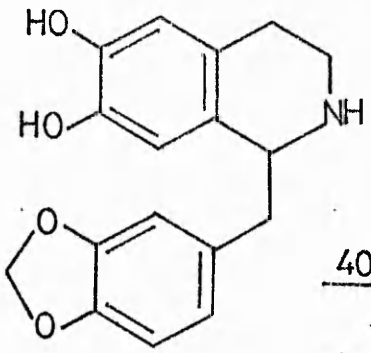
The aporphinoids consist of the aporphine, proaporphine, neoproaporphine, oxoaporphine and homoaporphine alkaloids. Aporphine alkaloids have the skeletal structure 43 and are the largest single group of naturally occurring alkaloids. Synthetic routes to such compounds will be discussed in Chapter 3. Extensive research using tracers has enabled the biogenesis of aporphine alkaloids to be classified into three distinct modes of phenol oxidative coupling.

a) Direct coupling, in which the relevant phenolic precursor possesses the correct substitution pattern to allow either ortho-ortho, ortho-para or (less commonly) para-para oxidative coupling to occur. Thus for example a compound having structure 44 can by ortho-ortho

41

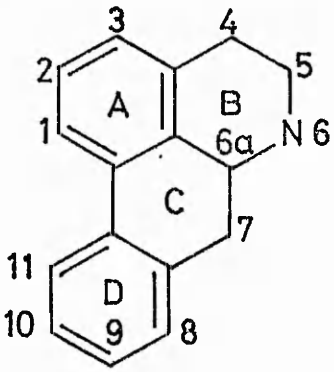


42

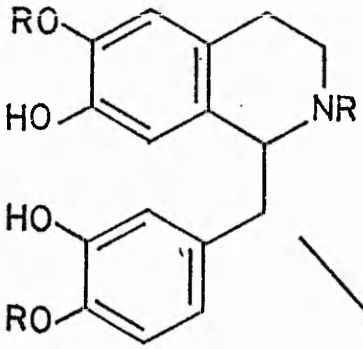


40

43

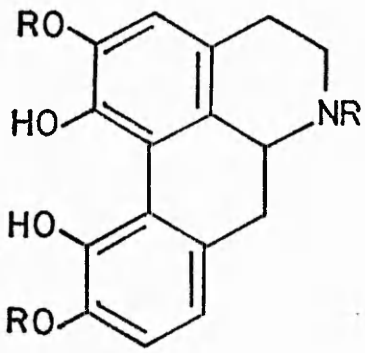


44

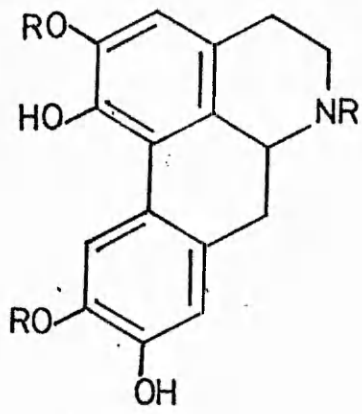


o-o

o-p



45



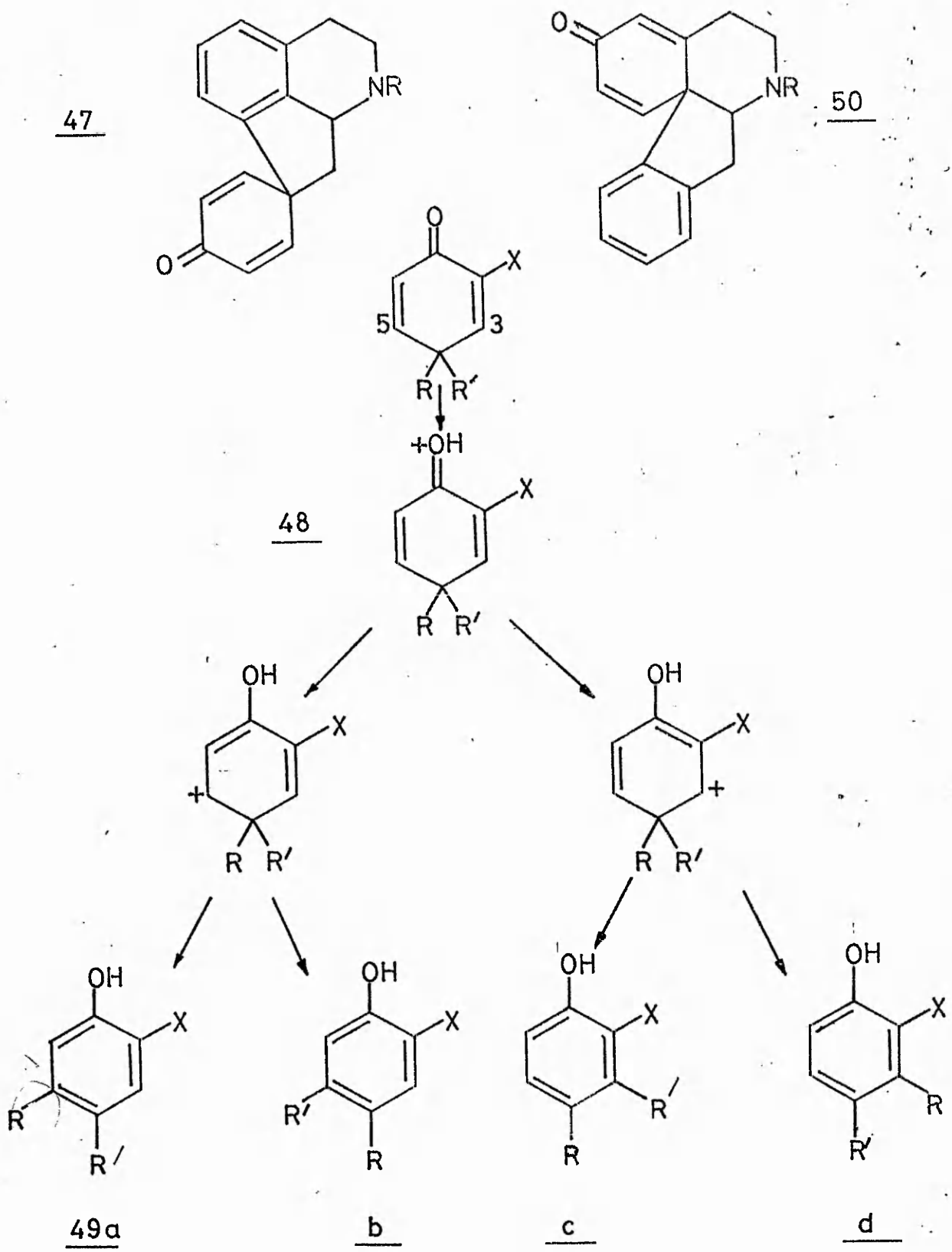
46

linkage produce 45, or by ortho-para coupling give 46.

b) Coupling to proaporphines, which have the skeletal structure 47, occurs when the phenolic moiety on ring D is of a different substitution type to that mentioned above, and consequently is unable to take part in direct coupling. Barton and Cohen[4] realised that alkaloids having this "wrong" substitution arrangement could, via the formation of dienone intermediates (later termed proaporphine alkaloids[72]), and subsequent rearrangement give the naturally occurring aporphines, which could not be explained in terms of a direct coupling reaction.

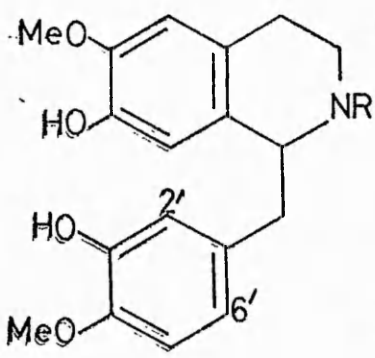
Two kinds of rearrangement can take place, viz. dienone-phenol[73] or -dienol-benzene. Both are acid catalysed carbonium ion transformations, with the former involving retention of the oxygen atom and the latter resulting in loss of this same function. Using the 2-substituted cyclohexa-2,5-dienone 48 as an example, dienone-phenol rearrangement can lead to four possible products 49a-d. The precise product formed cannot be predicted with certainty although the steric effect of a 2-substituent would direct rearrangement towards C-5 rather than C-3, as would an electronegative substituent. It has been demonstrated[74] that usually a more substituted alkyl group migrates more readily than a less substituted one, and that aryl groups migrate in preference to alkyl groups.[75-78] In all cases a carbon bond migrates more easily than a heteroatom bond.

c) Coupling to give the neoproaporphine structure 50 is similar to the previous mode of coupling, with the exception that the dienone function is formed on ring A rather than ring D. Similar rearrangements lead to aporphines having "unnatural" substitution patterns.

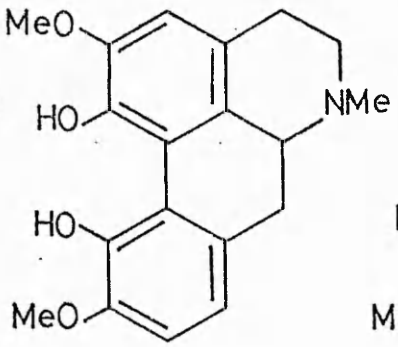

47
NR
O
50
NR
O
X
5
3
R
R′
+OH
X
48
R
R′
OH
X
+
R
R′
OH
X
+
R
R′
OH
X
R
R
OH
X
R′
R
OH
X
R
R′
OH
X
R
R′
49a
b
c
d

1.2.2 Direct Coupling to Give Aporphines

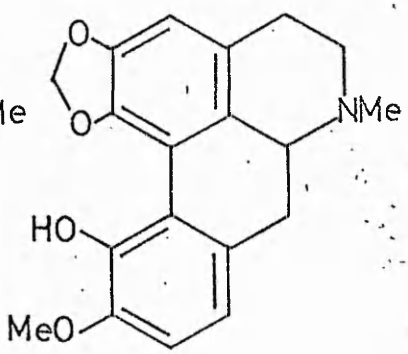
The first indication of such a coupling pathway came from the finding[79,80] that labelled reticuline 51 when introduced into Corydalis cava gave the appropriately labelled bulbocapnine 52, via the diphenol corytuberine 53, as would be predicted by direct ortho-ortho coupling. Reticuline has been isolated from this plant species by feeding and dilution experiments.[81] In order to eliminate an alternative pathway involving initial C-6′ hydroxylation of reticuline 51, followed by dienone formation and subsequent rearrangement as shown in Scheme V, C-2′ and C-6′ tritium labelled reticuline was fed to C. cava. The resulting bulbocapnine 52 was found to have retained the necessary amount of activity at the position corresponding to C-6′ and to have lost the C-2′ activity, consistent with ortho-ortho coupling. A study[82] of the biosynthesis of boldine 54 in Litsea glutinosa showed that (+) reticuline was stereospecifically incorporated whereas other possible phenolic precursors capable of operating via a dienone mechanism were not, demonstrating that direct ortho-para coupling is the preferred pathway to this alkaloid; initially isoboldine 55 is produced followed by methylation - demethylation stages to achieve the correct substitution pattern. Reticuline has also been implicated[83,84] in the biosynthesis of isoboldine in Papaver somniferum, whereas neither orientaline 56 nor N-norprotosinomenine 57 were found to be incorporated; once again the inference is that direct coupling rather than formation of either proaporphine or neoproaporphine is the preferred route. In the Aquilegia species biosynthesis of the quaternary aporphine, magnoflorine 58 has been demonstrated[85] to involve ortho-ortho coupling of reticuline, in contrast to the findings from P. somniferum in which reticuline was not incorporated into 58 to any significant extent.[83]



51 R=Me
112 R=H

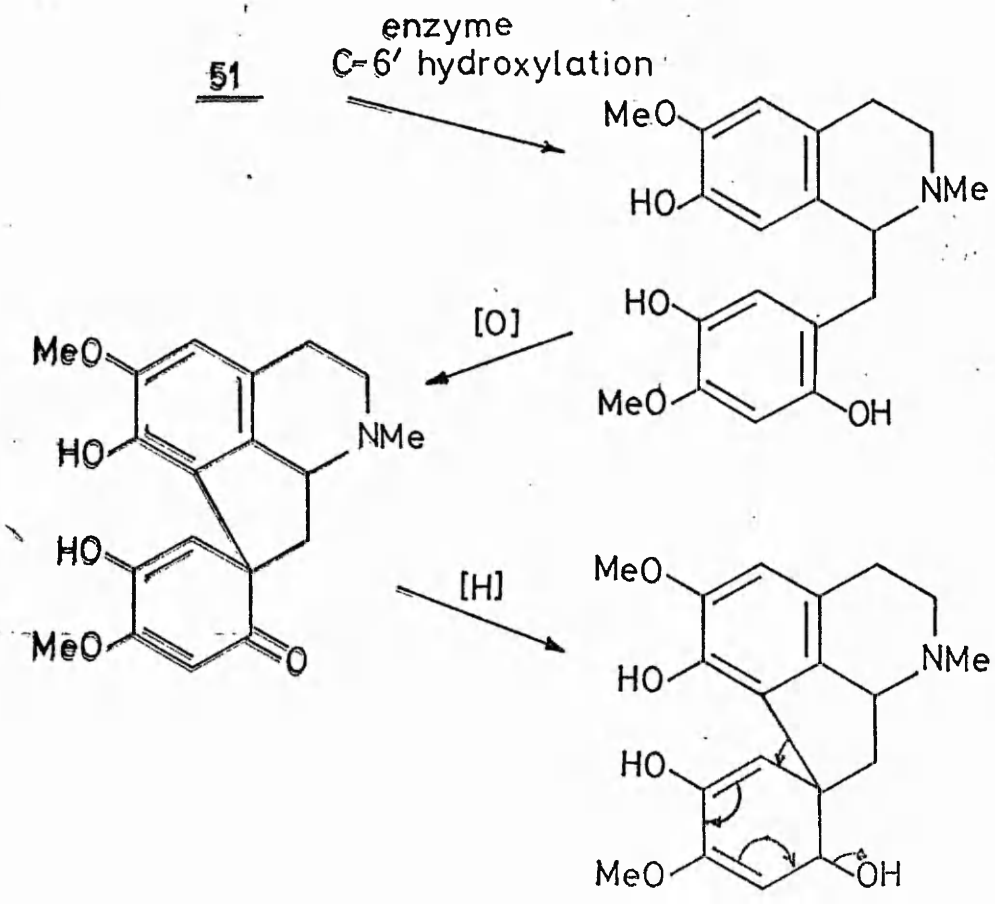


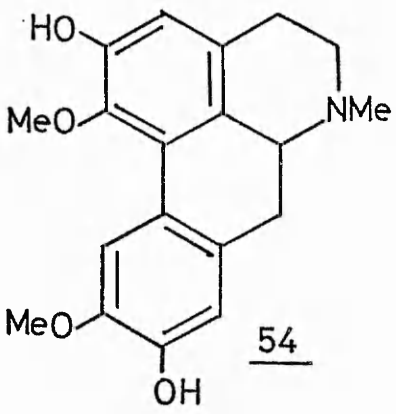
53



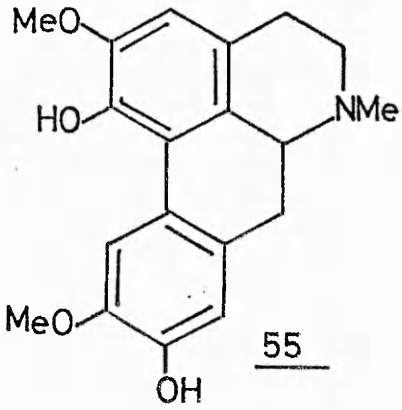
52

SCHEME V

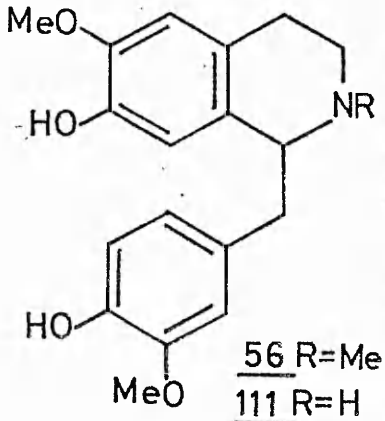




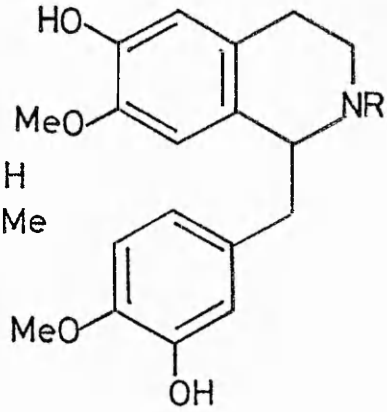
54



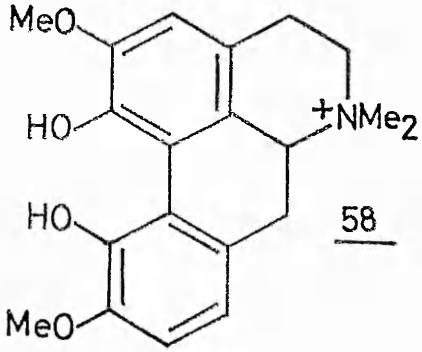
55



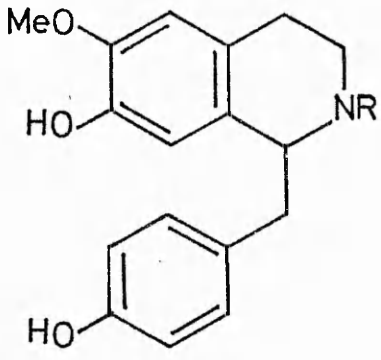
56 R=Me
111 R=H



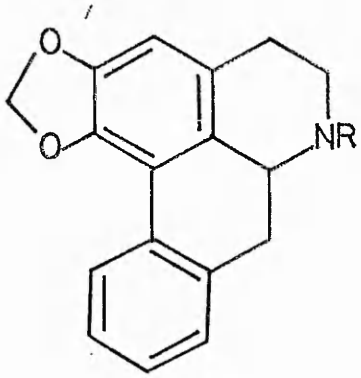
57 R=H
103 R=Me



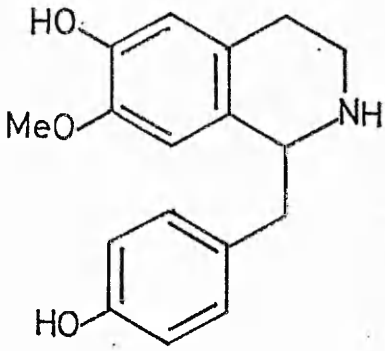
58



59 R=Me
64 R=H



60 R=Me
65 R=H

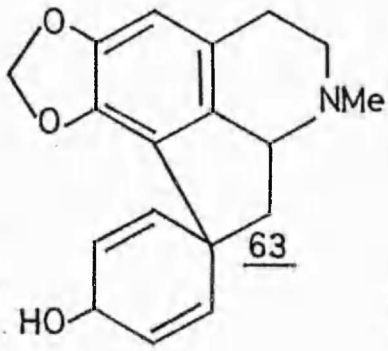


61

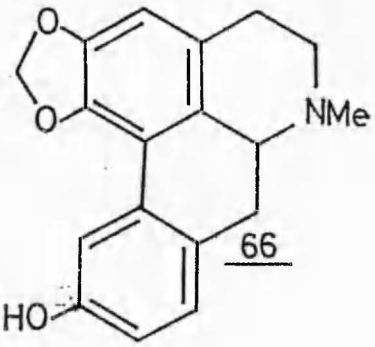
1.2.3 Coupling to Give Proaporphines

The involvement of proaporphines as intermediates in the biogenesis of aporphine alkaloids was postulated[4] prior to actual isolation of such compounds in plants. This postulate has been proved by both the isolation of such dienone compounds and their conversion into the predicted aporphines. Barton and his co-workers[86] demonstrated that (+) N-methylcoclaurine 59 is stereospecifically incorporated into roemerine 60 in Papaver dubium. Neither (-) N-methylcoclaurine nor isococlaurine 61 which lacks the appropriately situated phenolic function, were incorporated. The final product, i.e. 60, had therefore lost the hydroxy group on ring D; this was explained in terms of initial formation of the dienone 62 followed by reduction to a dienol 63 with subsequent rearrangement. In other experiments it was established that coclaurine 64 was converted into (-) anonaine 65 in Anona reticulata,[86,87] and into (+) mecambrinol 66 in Meconopsis cambrica,[88] both via proaporphine intermediates with the former being produced by the dienol-benzene rearrangement of 63, and the latter by the dienone-phenol transformation of 67. (±) Orientaline 56 has been demonstrated[89,90] to be a precursor in Papaver orientale of (±) isothebaine 68, which is formed via the intermediates orientalinone 69 and orientalinol 70, accompanied by the appropriate rearrangement of the latter; of the two epimeric structuresfor 70 only one was found to be efficiently incorporated. More recent work[91] has shown coclaurine 64, but not isococlaurine 61 to be a precursor in the biogenesis of the proaporphine and aporphine alkaloids, crotosparine 71, crotosparinine 72, and sparsiflorine 73 found in Croton sparsiflorus. An interesting feature of these alkaloids is that 72 has the opposite configuration at the C-1 position to the other two, suggesting that its biogenesis occurs by utilisation of the opposite enantiomer of coclaurine. In

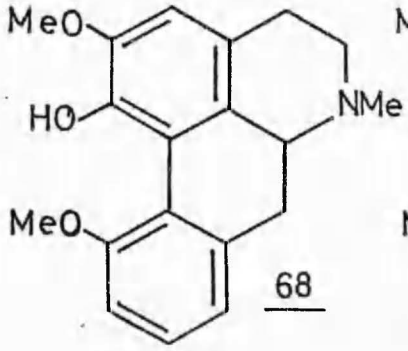
62 R=H
67 R=Me



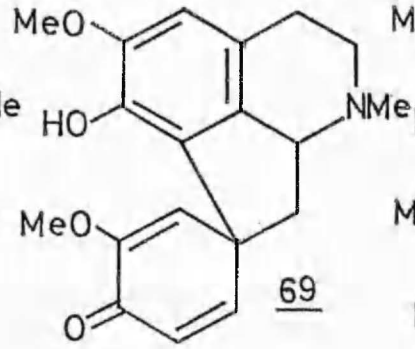
63



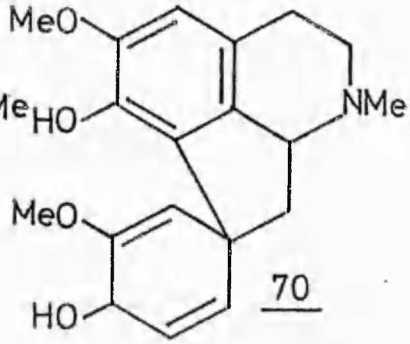
66



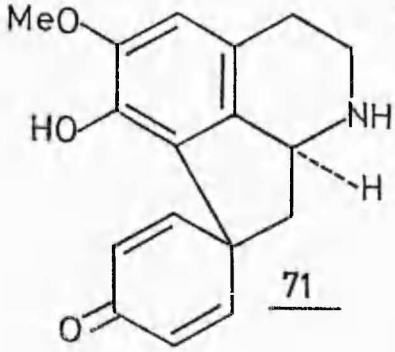
68



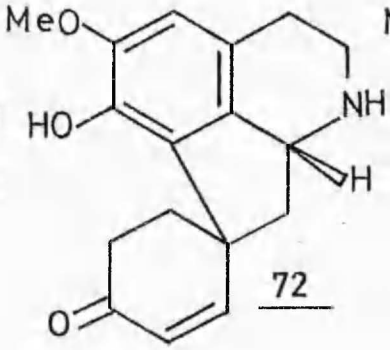
69



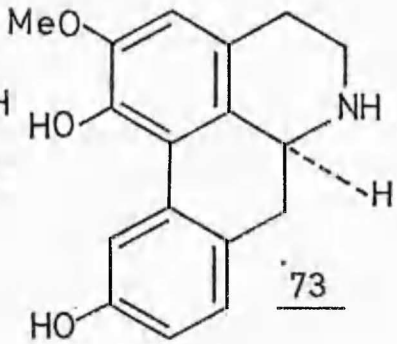
70



71

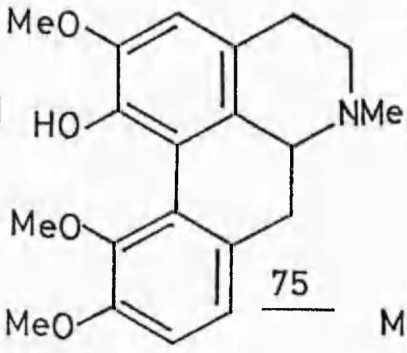


72

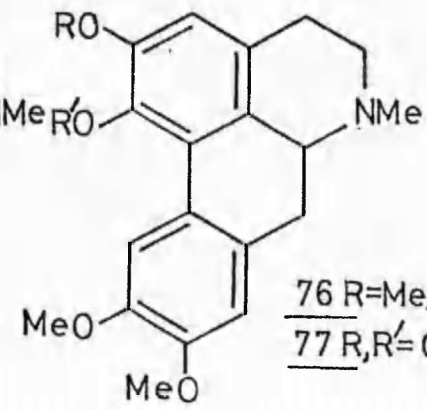


73

74



75



76 R=Me, R'=Me
77 R, R'= CH_2

addition, trapping experiments have shown coclaurine to be present in C. sparsiflorus. Similarly crotonosine 74 has been shown to be derived from (+) coclaurine in Croton linearis plants.[92,93]

1.2.4 Coupling To Give Neoproaporphines

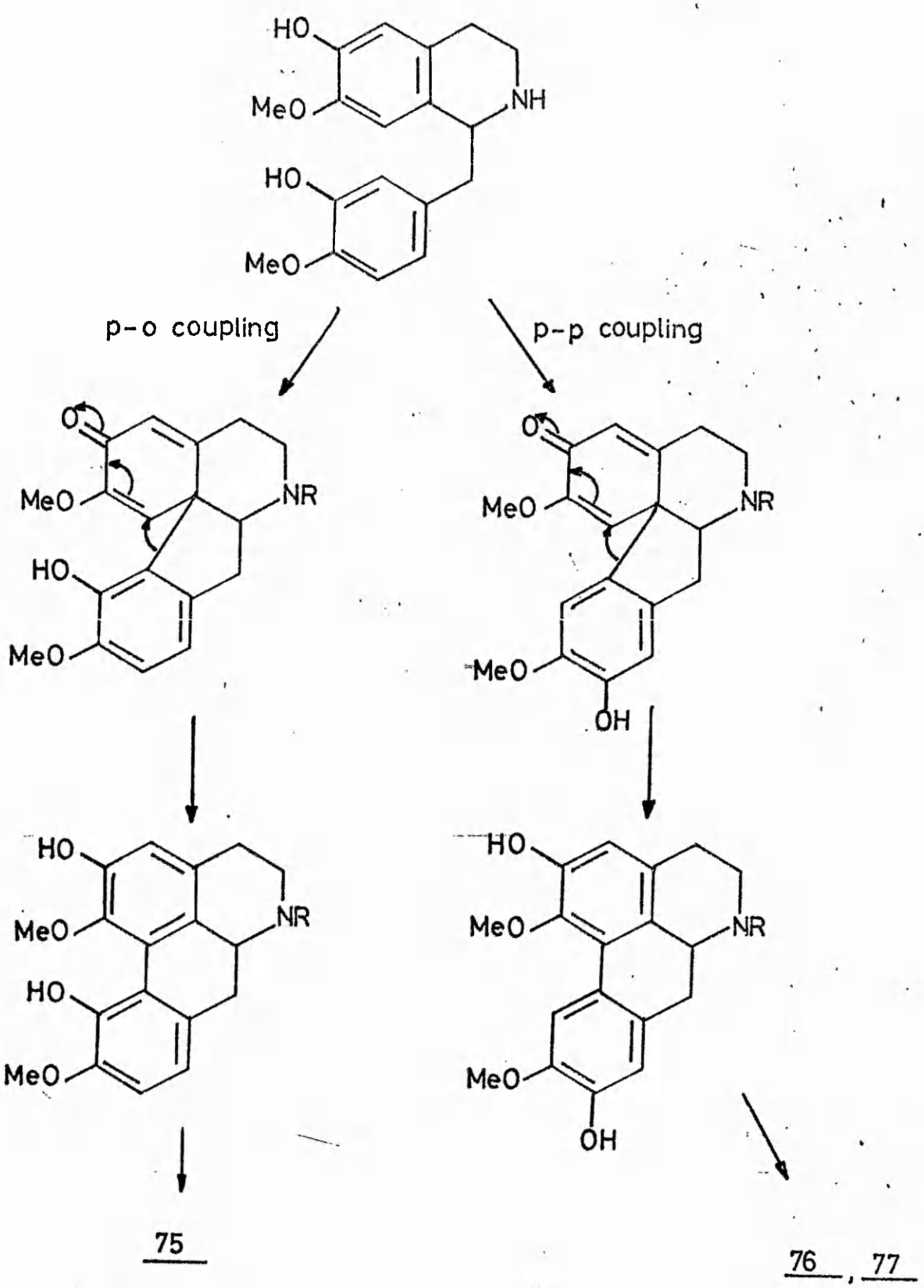
Battersby and his collaborators[94] have studied the biosynthesis of the Dicentra eximia aporphine alkaloids, corydine 75, glaucine 76 and dicentrine 77. Their findings implicate norprotosinomenine 57 as a key intermediate, even though the methylation pattern of 75 would strongly infer the involvement of either reticuline 51 or orientaline 56, (neither of which were incorporated in feeding studies). The finding that 57 was an efficient precursor gave rise to the theory that a dienone-phenol rearrangement of a neoproaporphine type intermediate was involved in the formation of 75, 76 and 77 as illustrated in Scheme VI.

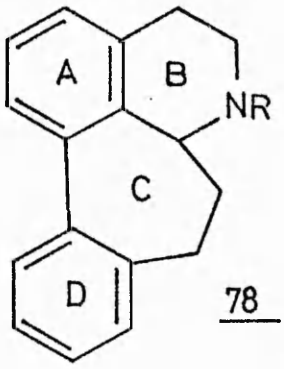
Unlike the proaporphine series, no neoproaporphine has as yet been isolated and assuming that these compounds do exist it would seem likely that they can be reduced to the appropriate dienol compound, to give after rearrangement an aporphine having an unsubstituted C-2 position. To date no naturally occurring aporphine alkaloids are known which possess this hydroxylation pattern in ring A.

1.2.5 Homoaporphines

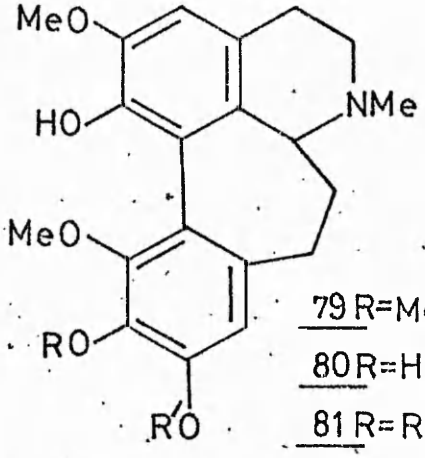
Homoaporphines have the skeleton structure 78. Alkaloids of this type, e.g. floramultine 79, multifloramine 80 and kreysigine 81 have been isolated from the species Kreysigia multiflora. Labelling studies have shown[95] that autumnaline 82 was well incorporated into these

SCHEME VI

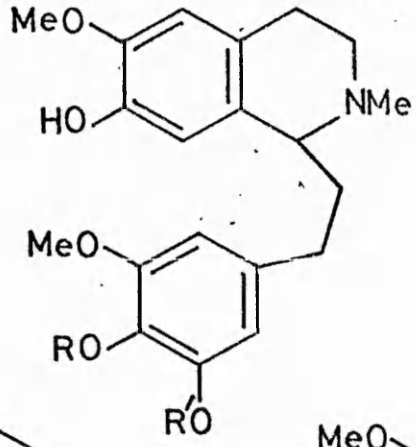

HO
MeO
NH
HO
MeO
p-o coupling
p-p coupling
O
MeO
NR
HO
MeO
O
MeO
NR
MeO
OH
HO
MeO
NR
HO
MeO
HO
MeO
NR
MeO
OH
75
76 , 77



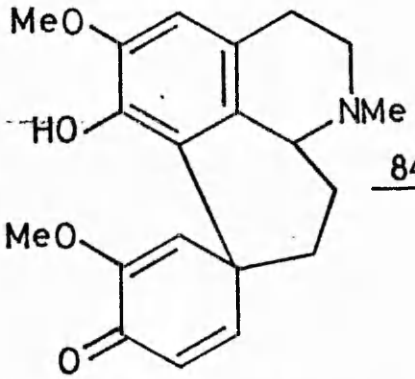
78



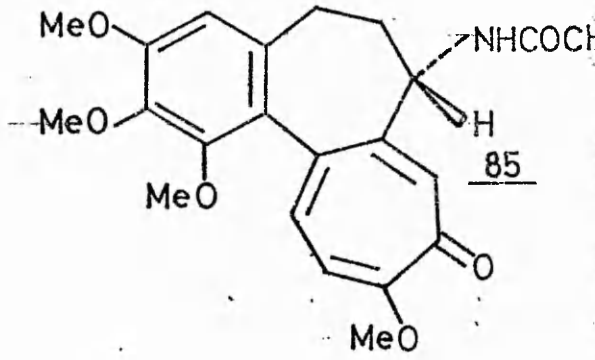
79 R=Me, R'=H
80 R=H, R'=Me
81 R=R'=Me



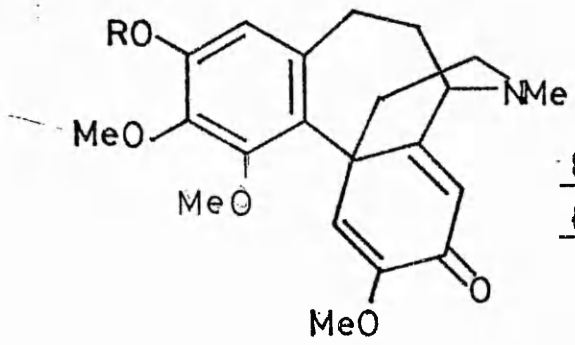
82 R=Me, R'=H
83 R=H, R'=Me



84



85



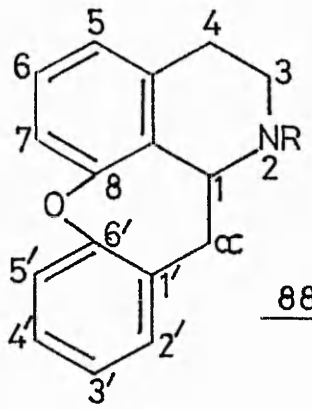
86 R= H
87 R=Me

alkaloids in contrast to its isomer 83 which did not become involved to any significant extent, pointing to a direct phenol coupling process for such alkaloids, even though kreysiginone 84 has been found in K. multiflora.[96]

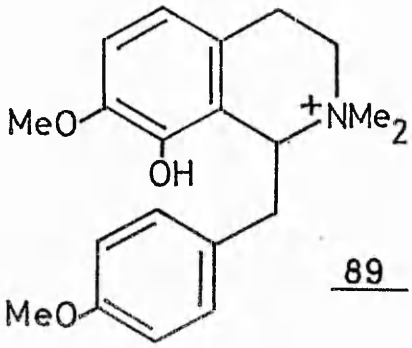
The phenylethylisoquinoline autumnaline 82 has also been postulated[97] as being a precursor of colchicine 85, which although not an alkaloid by strict definition, since it has a non-basic nitrogen, is generally regarded as such. Direct para-para coupling of autumnaline produces the dienone intermediate 86, followed by methylation to give another dienone, O-methylandrocymbine 87 which has been incorporated into colchicine to a remarkably high degree. More recently ^{13}C nmr work has confirmed this pathway.[98]

1.2.6 Cularine Alkaloids

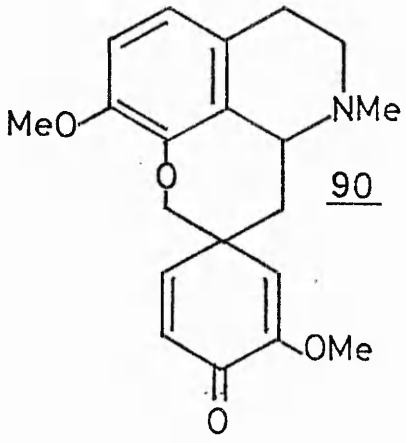
There are just four naturally occurring members of the cularine alkaloid family based upon the structure 88. The alkaloids of this group are unique in that they possess the rare 7,8-oxygenation pattern in addition to a dihydro oxepin ring system. The synthetic methods for preparing cularine based alkaloids are discussed in Chapter 4. To date no work on the biosynthesis of this class of alkaloids has been reported; however three routes appear possible as illustrated in Schemes VII, VIII and IX. Because of this unusual 7,8-substitution pattern it was not until the finding of petaline 89 in Leontice leontopetalum[99] that a suitable naturally occurring precursor was apparent, although many workers[4,99,100] had postulated the involvement of such a compound. An in vitro study[101] of the isomeric dienone intermediates 90 and 91 failed to give the appropriate cularine compounds by rearrangement, in contrast



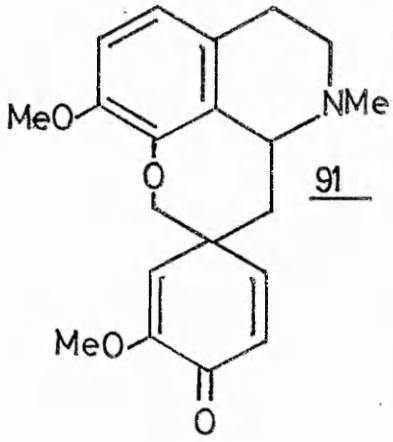
88



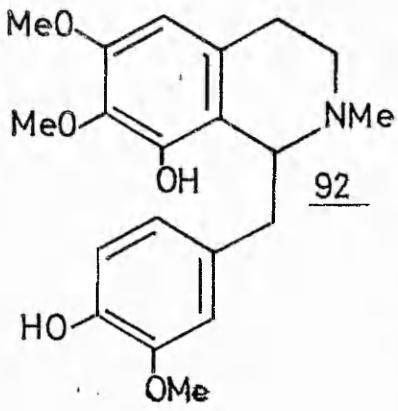
89



90

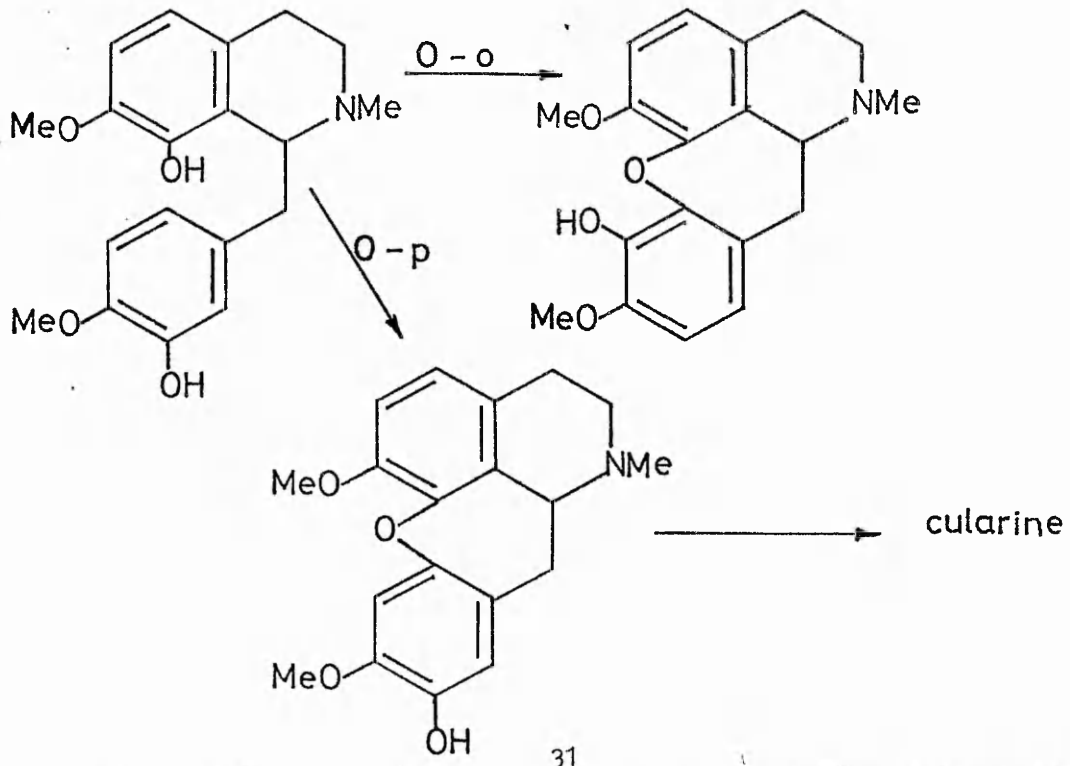


91

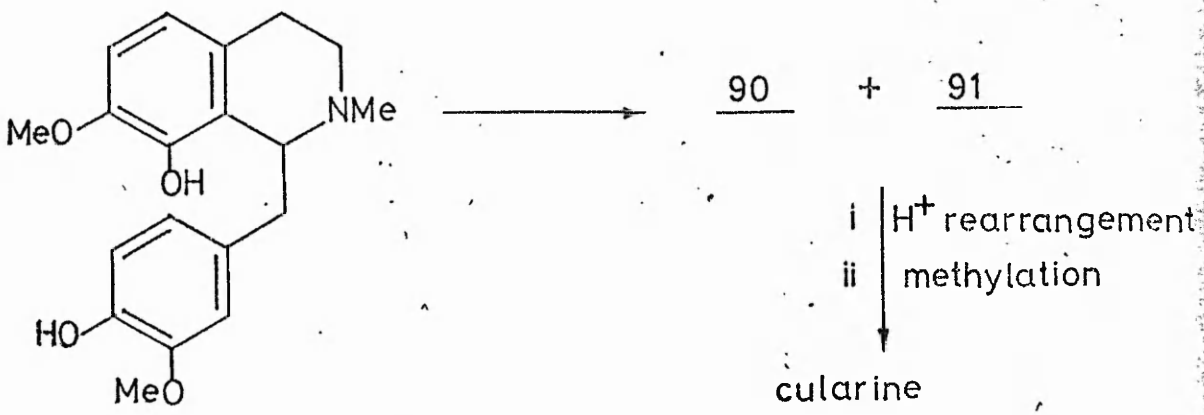


92

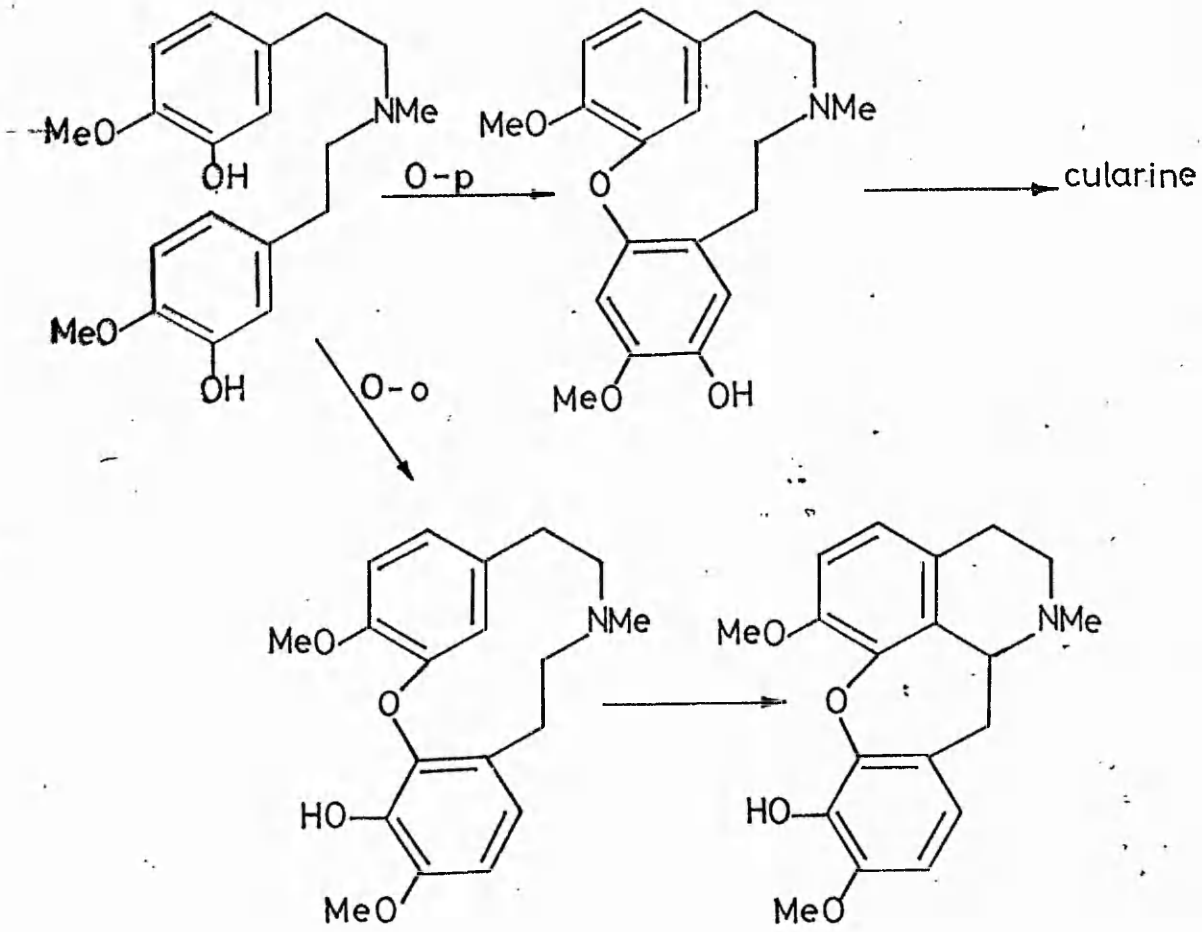
SCHEME VII



SCHEME VIII



SCHEME IX



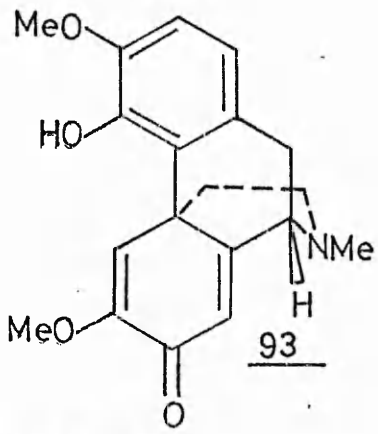
to the ferricyanide oxidation of 92 which gave low yields of an unnatural cularine alkaloid via acid catalysed rearrangement of the appropriate dienone.[102]

1.2.7 Morphine Type Alkaloids

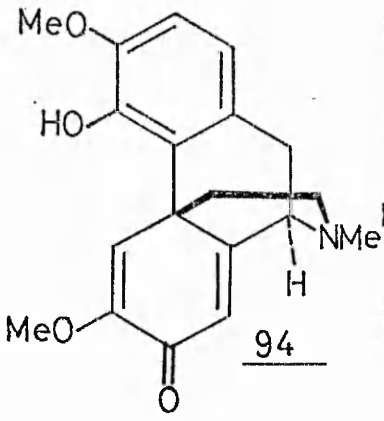
This class of alkaloids can be subdivided into two groups which bear an enantiomeric relationship to one another, e.g. sinoacutine 93 and salutaridine 94. In addition to the coupling modes of reticuline previously discussed in connection with the aporphine alkaloids, other linking schemes for the aryl units are possible, as shown in Scheme X, leading to a variety of products.

1.2.8 Morphine Alkaloids

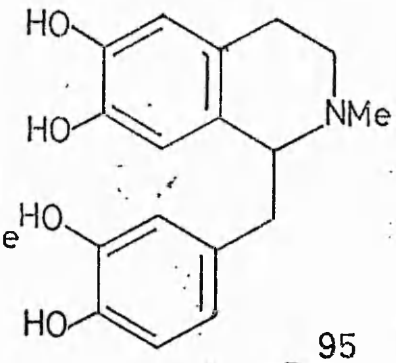
It has been established that only reticuline of the four possible dihydroxy-dimethoxy-1-benzylisoquinolines derived from laudanosoline 95 is incorporated into the opium alkaloids by Papaver somniferum.[103-106] An interesting feature of this work, using resolved (+) and (-) reticuline labelled with tritium and ^{14}C atoms, was the incorporation of both into morphine to almost the same extent with respect to ^{14}C activity. This is surprising since (-) reticuline has been shown[104] to correspond in absolute configuration with the morphine group.[107,108] However it was observed that with the (+) enantiomer almost complete loss of C-1 tritium activity occurred, as opposed to approximately 40% loss with the (-) form. This difference was explained[104] in terms of an interchange between the two enantiomeric forms via a 1,2-dehydro-reticuline 96, with the (-) form being incorporated directly into thebaine 97 while the (+) form was transformed into the (-) enantiomer through



93

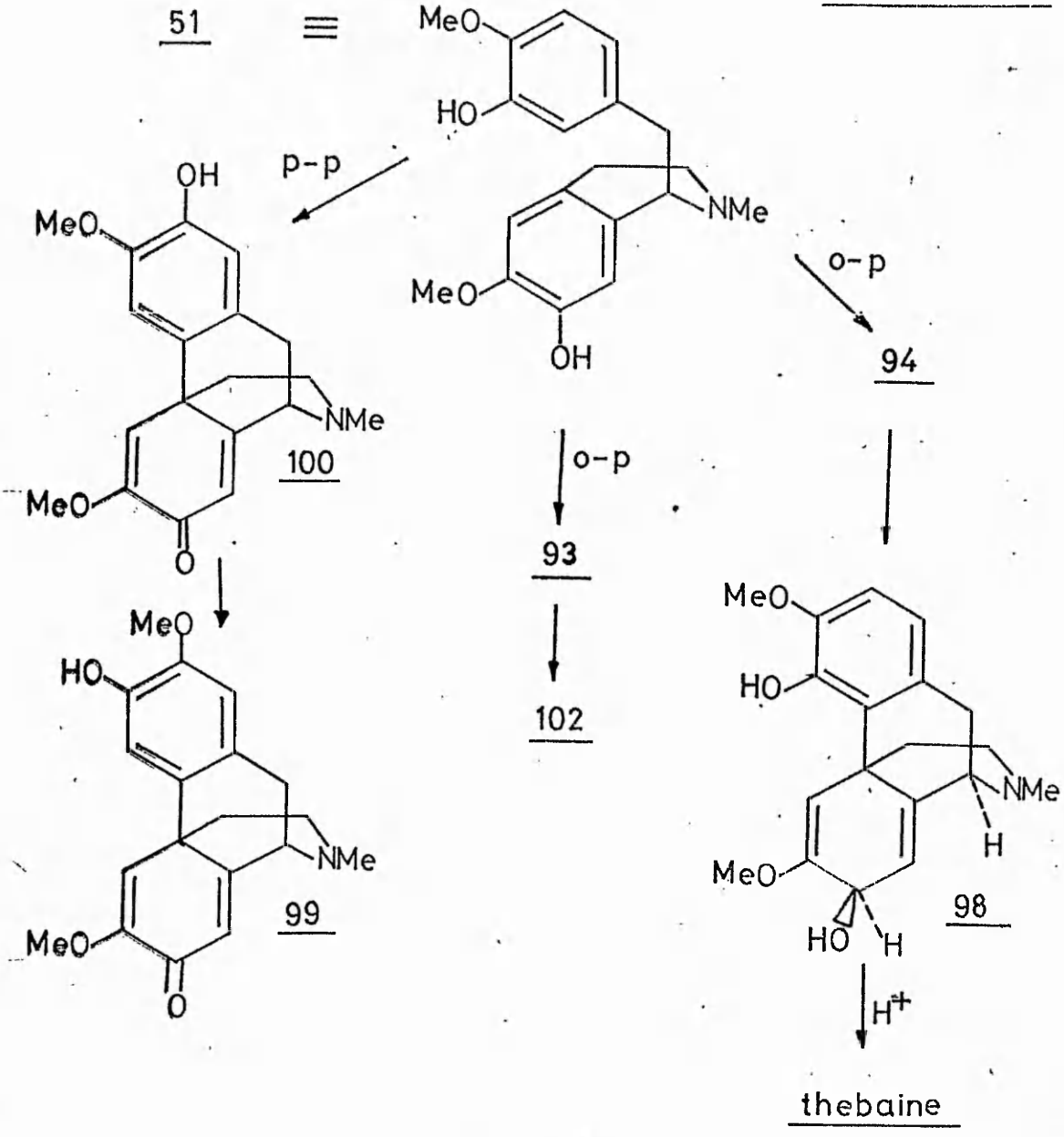


94



95

SCHEME X



96 with subsequent loss of tritium activity. This theory was confirmed[104,106] by the high and specific incorporation of labelled 1,2-dehydroreticuline 96 into morphine by P. somniferum plants. Reticuline has also been incorporated into thebaine in P. orientale, in addition to which 51 has, along with salutaridine 94, been detected in small amounts in opium and in adult poppy plants[109,110].

The product from phenol oxidative coupling of reticuline leading to the morphine alkaloids involves initial ortho-para linking to give the morphinandienone, salutaridine 94, which itself has been efficiently introduced into thebaine 97, codeine and morphine.[106] The biogenesis of 97 involves the reduction of 94 to salutaridinol-I 98, and it has been demonstrated that this particular epimer, with the hydroxy group of the dienol cis to the ethylamine bridge, is incorporated to a much greater extent than the alternative salutaridinol-II. Dehydration of the dienol produces thebaine via nucleophilic attack of the phenol grouping on the dienol system.

Para-para coupling of reticuline leads to another morphinandienone-type alkaloid, flavinantine 99 through the intermediacy of isosalutaridine 100 with subsequent demethylation-remethylation. Studies using Croton flavens[111] have demonstrated that reticuline is indeed a precursor of this particular morphinandienone 99. Furthermore it was observed that although coupling of reticuline is the probable major pathway to 99, a minor route involving para-para coupling of orientaline 56 to the bisdienone 101 followed by rearrangement also contributes to the biosynthesis of this alkaloid.[112]

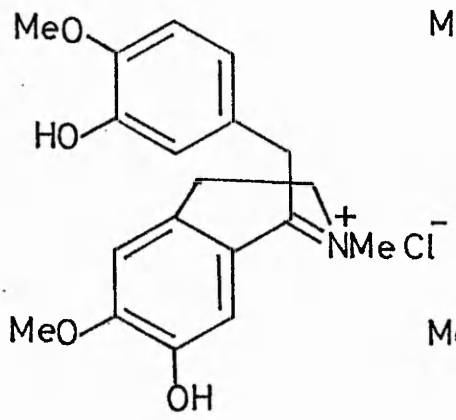
1.2.9 Sinomenine, Protostephanine and Hasubanonine

The alkaloid sinomenine 102 bears an antipodal relationship to morphine, with (+) reticuline being identified as the biological precursor[113,114] and not protosinomenine 103 as earlier proposed by Robinson.[115] Feeding experiments with Sinomenium acutum have shown incorporation of both reticuline and sinoacutine 93, the enantiomer of salutaridine, into 102.[114] Thus para-ortho coupling of (+) reticuline is the preferred pathway to sinomenine.

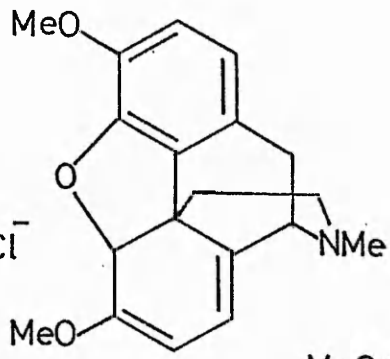
The biosynthesis of the alkaloids protostephanine 104 and hasubanonine 105, found in Stephania japonica plants, has been studied by Battersby and his co-workers.[116,117] Feeding with likely phenolic 1-benzylisoquinoline and N,N-bisphenylethylamine precursors has established that only those compounds having the general structure 106 are efficiently incorporated by S. japonica into 104 and 105. In view of this evidence a biosynthetic pathway to these alkaloids was proposed, and is illustrated in Scheme XI. Both protostephanine and hasubanonine are therefore "disguised" members of the 1-benzylisoquinoline group and appear to be unique in requiring two phenolic hydroxy groups in one of the rings involved in phenol oxidative coupling.

1.2.10 Erythrina Alkaloids

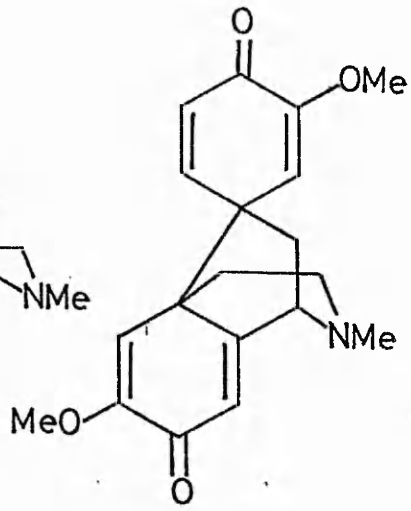
The proposed pathway to the erythrina alkaloids, typified by erythraline 107, is illustrated in Scheme XII. Para-para phenol coupling of the 1-benzylisoquinoline N-norprotosinomenine 57 gives the neoproaporphine-type compound 108 followed by ring cleavage and reduction to the symmetrical biphenyl 109. Biogenesis of erythrina alkaloids is therefore yet another variation on the 1-benzylisoquinoline-phenol oxidative coupling theme. Two groups[118,119] have demonstrated



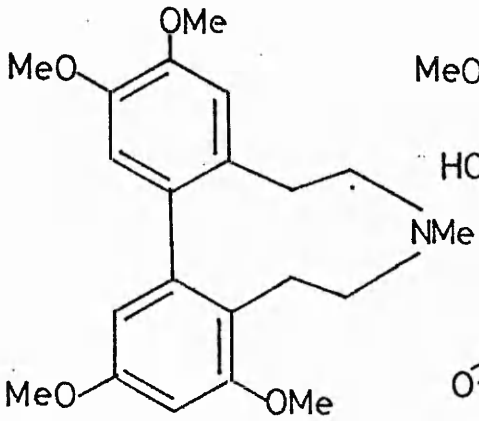
96



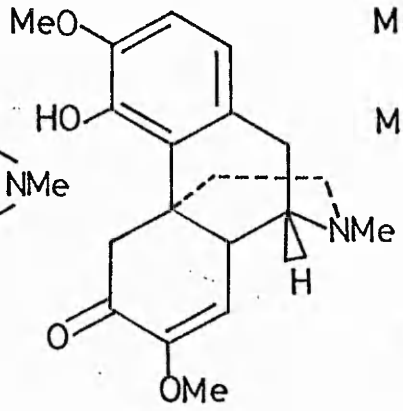
97



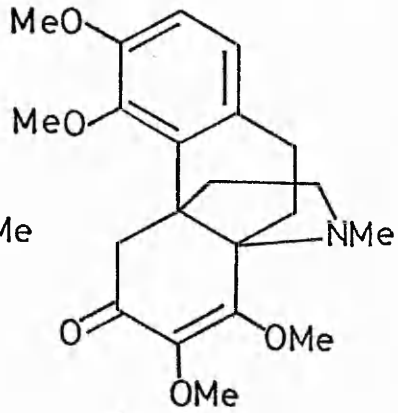
101



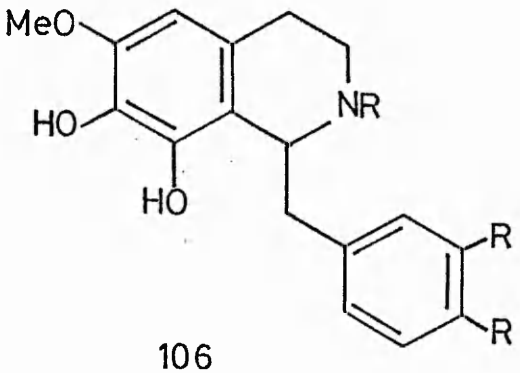
104



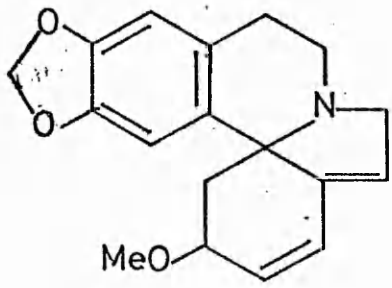
102



105



106



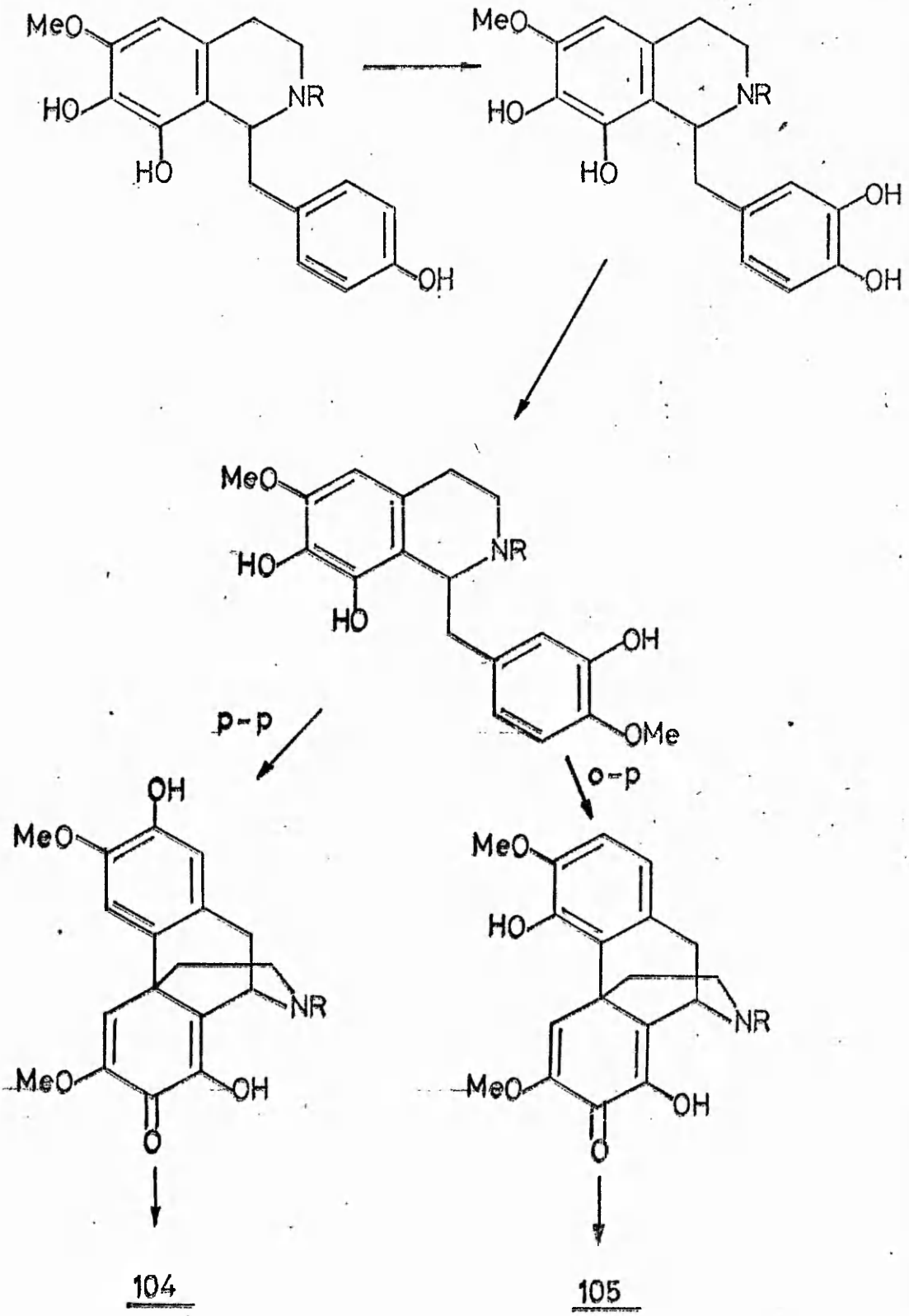
107

by feeding labelled (+) and (-) N-norprotosinomenine 57 to Erythrina crista galli that only the (+) enantiomer is significantly incorporated into erythraline 107 and erythratine 110. Similarly dibenzazonine 109 also gave good incorporation into the same compounds. The involvement of 109 was further indicated when [4'-methoxy-C^{14}]-N-norprotosinomenine was fed to E. crista galli, the isolated erythraline having an equal distribution of activity between the methoxy- and methylenedioxy-groups, clearly implicating a symmetrical intermediate along the pathway. It has recently been demonstrated[120] that although N-nororientaline 111 is present in Erythrina species, neither it nor N-norreticuline 112 was incorporated into erythraline 107 under conditions in which N-norprotosinomenine was an efficient precursor. It therefore seems from the available evidence that 57 is an exclusive precursor for this group of alkaloids. (-) Erysodienone 113 has also been demonstrated as a precursor of erythraline.[121] Two alternative pathways to the so called "abnormal" erythrina alkaloids, i.e. those having a single oxygen function at C-15 as opposed to the "normal" type which are oxygenated at both C-15 and C-16, both involving a dibenzazonine intermediate have been suggested.[70]

1.3 The Significance of Isoquinoline-1-Carboxylic Acids In Isoquinoline Alkaloid Biogenesis

It is now generally accepted[122,123] that the in vivo condensation of a β-phenylethylamine derived from either tyrosine or dopa, with the appropriate carbonyl compound is a key step in the formation of the isoquinoline alkaloids. The exact nature of this carbonyl moiety has long been the subject of much argument, resulting in three different mechanisms being proposed for this condensation reaction; these mechanisms are shown in Scheme XIII.

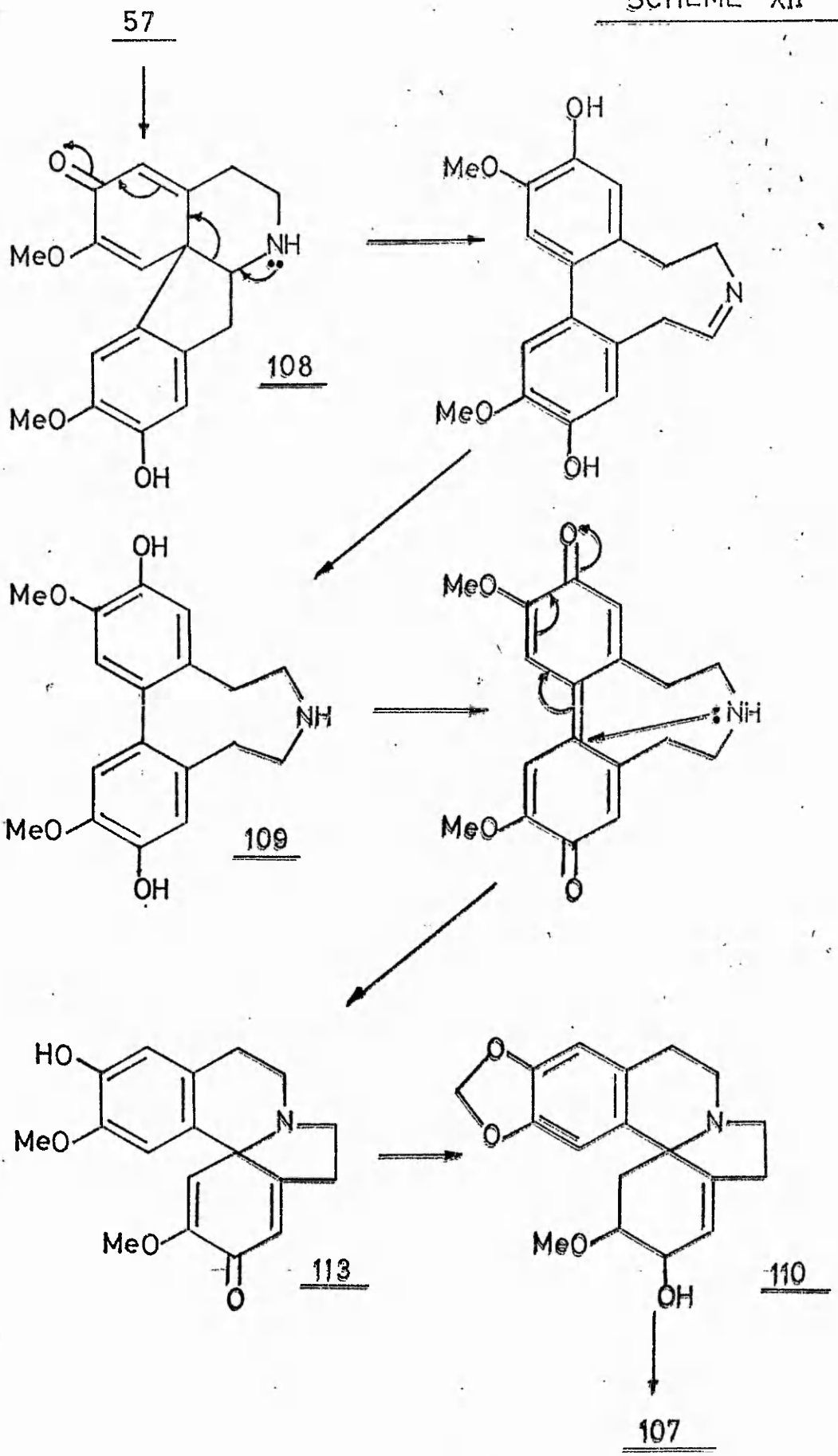
SCHEME XI



104

105

SCHEME XII


57
O
MeO
NH
MeO
OH
108
OH
MeO
N
MeO
OH
OH
MeO
NH
MeO
OH
109
O
MeO
NH
MeO
O
HO
MeO
N
MeO
O
113
O
O
N
MeO
OH
110
107

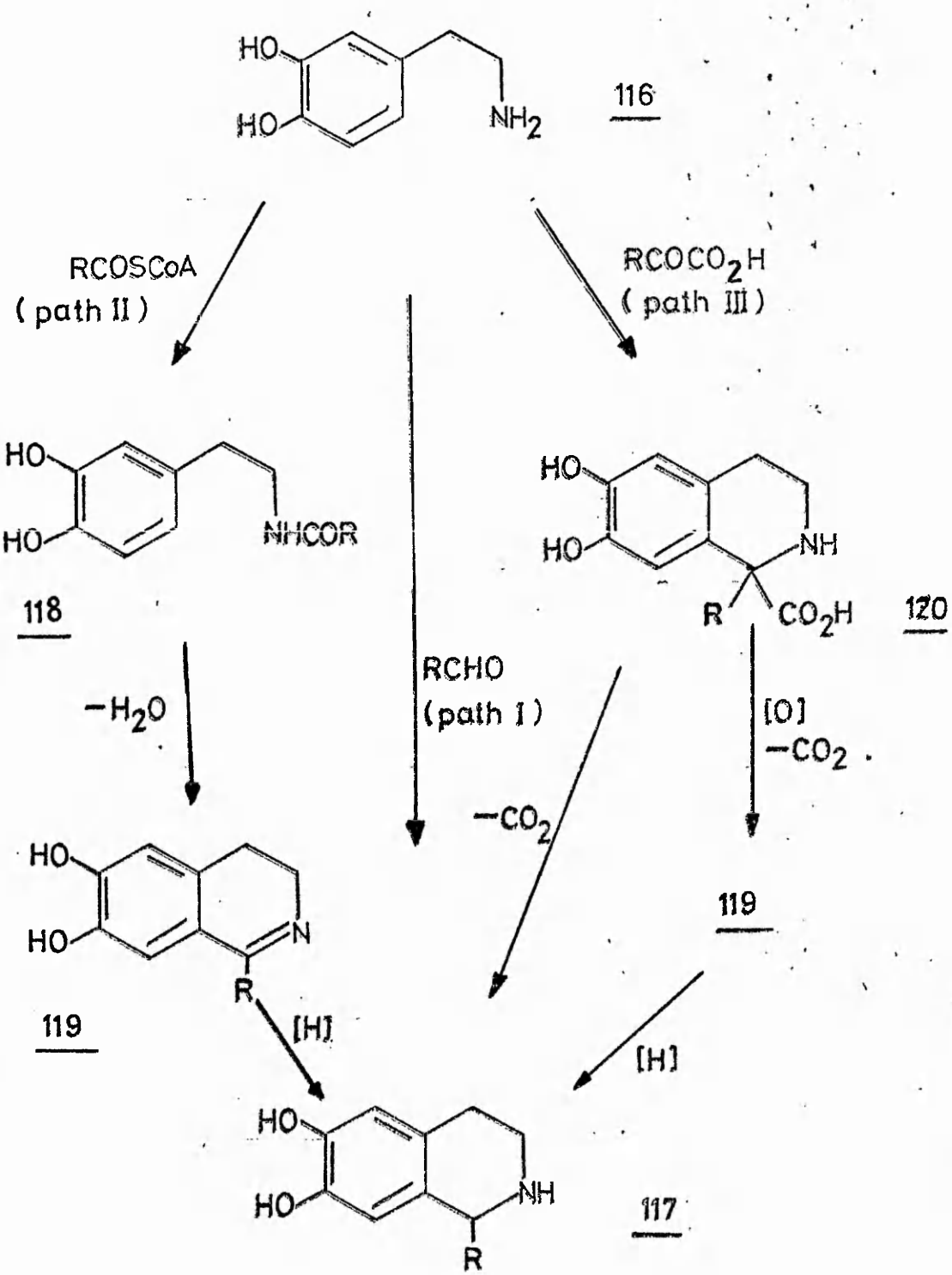
In 1911 Pictet and Spengler[124] found that condensation of formaldehyde with β-phenylethylamines gave, under physiological conditions, alkaloids of the anhalamine-type 114, suggesting that the source of the C-1 atom of tetrahydroisoquinolines was a simple aldehyde. Other workers[125,126] found that acetaldehyde gave rise to salsolidine-type alkaloids 115 and that substituted phenylacetaldehydes gave 1-benzylisoquinolines under similar conditions. This route, utilising the carbonyl function of an aldehyde, is represented by path I, i.e. 116 to 117 in Scheme XIII. Little evidence is available for the involvement of such a mechanism in nature, since aldehydes are generally regarded as being too reactive to be readily available in plants. An exception, however, appears to be the synthesis of the indole alkaloids of Vinca rosea, in which the formyl function of secologanin 121 has been shown[127] by tracer studies to be the participating carbonyl group. Both tyrosine and dopa have been demonstrated to be the main building molecules for several 1-benzylisoquinoline alkaloids, with dopa only being incorporated into the phenylethylamine half of such molecules.[128-130] It would seem reasonable to expect, that if the necessary phenylacetaldehyde was derived from dopa via dopamine and a deamination reaction, incorporation into the bottom half of the molecule should also occur.

A second mechanism (path II) involves[131] initial acylation of a β-phenylethylamine 116 (e.g. by acetylcoenzyme A in the case of the salsolidine alkaloids), followed by cyclisation of the resulting amide 118 to give 119. The incorporation of N-acetyltryptamine into the indole alkaloid harman 122 has been offered as evidence for this mechanism.[132] However, other workers[133,134] have found that N-acetyl phenylethylamine was initially deacetylated prior to being incorporated into the peyote alkaloids. Similarly it was found[135] that N-acetyl-3-

hydroxy-4-methoxyphenylethylamine was not incorporated into the alkaloid salsolidine 115. The validity of this mechanism is therefore in doubt.

An alternative proposal put forward by Hahn[2] involved the intermediacy of an isoquinoline-1-carboxylic acid 120 (path III) which was formed by condensation of the amine 116 with an ∝-keto acid. Subsequent decarboxylation of 120 resulted in the formation of either a di- or tetrahydroisoquinoline depending upon the nature of such a decarboxylation step. A mechanism based upon this sequence was initially rejected following the failure of both Hahn[2] and Whalley and Govindachari[136] to decarboxylate acids of this type under mild conditions *in vitro*. More recently this mechanism had received considerable support from both feeding and isolation experiments in various plants. Pyruvate has been demonstrated to be an efficient precursor of both the peyote alkaloid[131,134] anhalonidine 123 and the β-carboline alkaloid[137] harmine 124, whilst the isoquinoline-1-carboxylic acids 125 and 126 have been detected in peyote cacti.[131,138] The most significant findings involve the incorporation of isoquinoline-1-carboxylic acids into several groups of alkaloid. Kapadia[133] found on feeding labelled peyoxylic and peyoruvic acid (125 and 126) to peyote cacti, that radioactive anhalonidine 123 and anhalamine 114 were produced, thus inferring that these acids are true intermediates along the biosynthetic pathway to such alkaloids. In addition it was observed that when fresh slices of the same cacti were used in place of the living plant, the 3,4-dihydroisoquinoline 127 was obtained from acid 126, leading to the proposal that an oxidative decarboxylation is involved. The more commonly found tetrahydroisoquinoline compounds

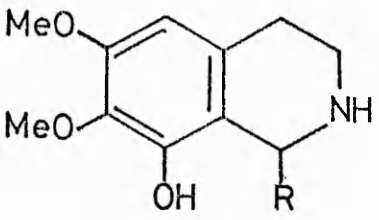
SCHEME XIII


HO
HO
NH2
116
RCOSCoA
(path II)
RCOCO2H
(path III)
HO
HO
NHCOR
118
HO
HO
NH
R
CO2H
120
RCHO
(path I)
−H2O
[O]
−CO2
−CO2
HO
HO
N
R
119
119
[H]
[H]
HO
HO
NH
R
117

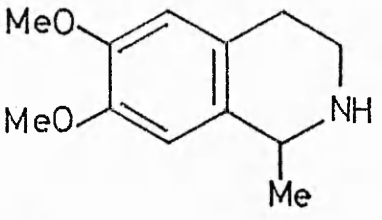
may be formed by subsequent reduction of the 3,4-dihydro species, e.g. by NADPH. Similarly acid 128 has been identified as a precursor of salsolidine 115 in Echinocereus merkeri.[139] Other workers have studied the role of the 1-benzyl acid 129 in Papaver orientale,[140] P. somniferum[141] and Litsea glutinosa.[130] In all cases this acid was found to be incorporated into the appropriate alkaloid, i.e. norlaudanosoline, morphine and reticuline respectively. It was also found that the dihydroisoquinoline 130 was efficiently incorporated into morphine[141] and reticuline[130] by P. somniferum and L. glutinosa, but that neither the triphenolic acid[130] 131 nor dihydroisoquinoline[141] 132 were, using either of these plants. The importance of isoquinoline-1-carboxylic acids in the biosynthesis of certain isoquinoline alkaloids has therefore been adequately demonstrated; the decarboxylation stage must now be considered.

1.3.1 Decarboxylation
1.3.2 Pyridoxal Mediated Reactions

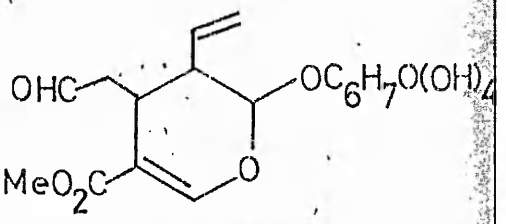
The significance of the decarboxylation of amino acids in biological processes has long been appreciated and has been adequately reviewed,[142] as have the amino acid decarboxylases.[143] Enzymic decarboxylation of α-amino acids is known to be an important process in the biosynthesis of alkaloids, especially the tetrahydroisoquinolines.[144] The detection of amines in media known to contain the corresponding α-amino acids implied[145] that enzymes capable of decarboxylating such acids were present in certain living tissues and micro-organisms. It was established[146,147] that for almost all such enzymes a cofactor, identified as a phosphorylated derivative of pyridoxal (later shown[148,149] to be the 5′phosphate 133) was essential in order for decarboxylation



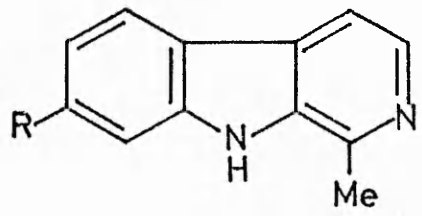
114 R=H
123 R=Me



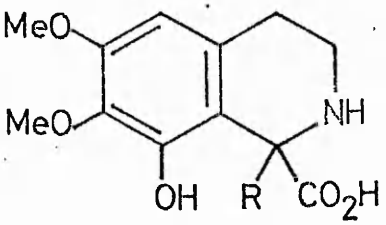
115



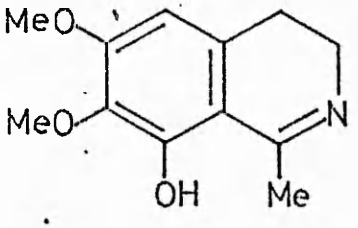
121



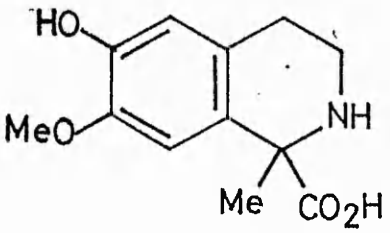
122 R=H
124 R=MeO



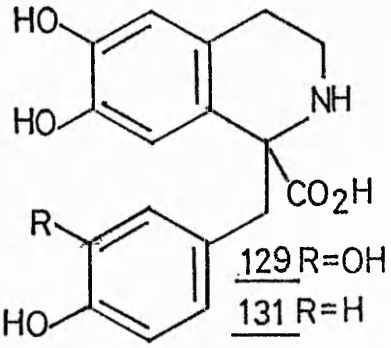
125 R=H
126 R=Me



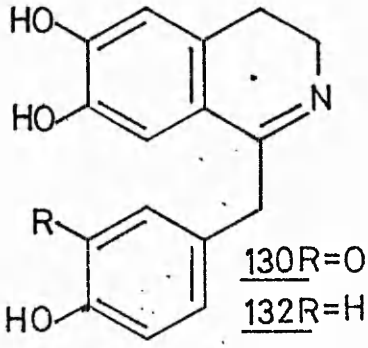
127



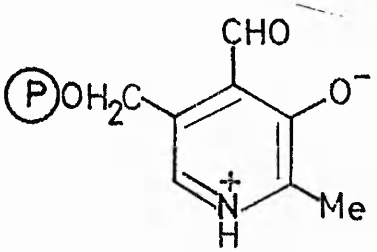
128



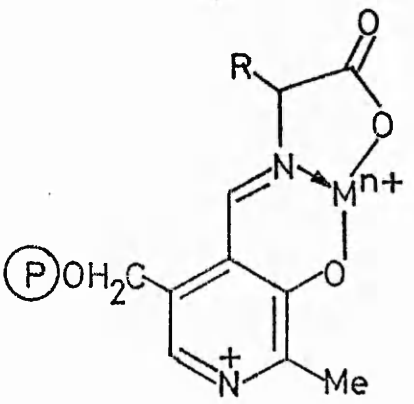
129 R=OH
131 R=H



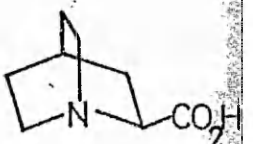
130 R=OH
132 R=H



133



135

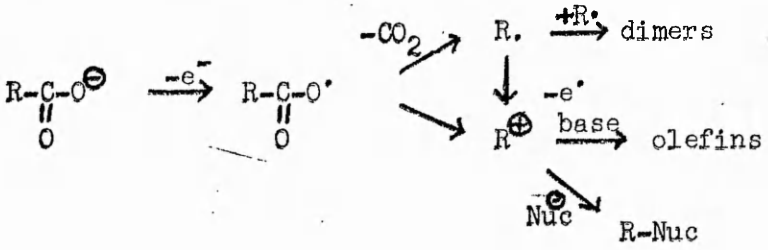


136

to take place. A mechanism for the reaction, involving the initial formation of an aldimine structure 134, was suggested by Metzler[150] and is shown in Scheme XIV. The formation of this aldimine was considered to be both catalysed and stabilised by the presence of certain metal ions capable of generating a metal-substrate complex of type 135. Such a structure as 134 possesses a highly conjugated system capable of delocalising the increased electron density which builds up at the carbon atom adjacent to the decarboxylation site, during the loss of the carboxy group. The nonenzymic pyridoxal-catalysed decarboxylation of α-aminoisobutyric acid resulted in the occurrence of both decarboxylation and a decarboxylation-dependent transamination,[151] suggesting that one function of the protein part of the enzyme is to eliminate this side reaction.

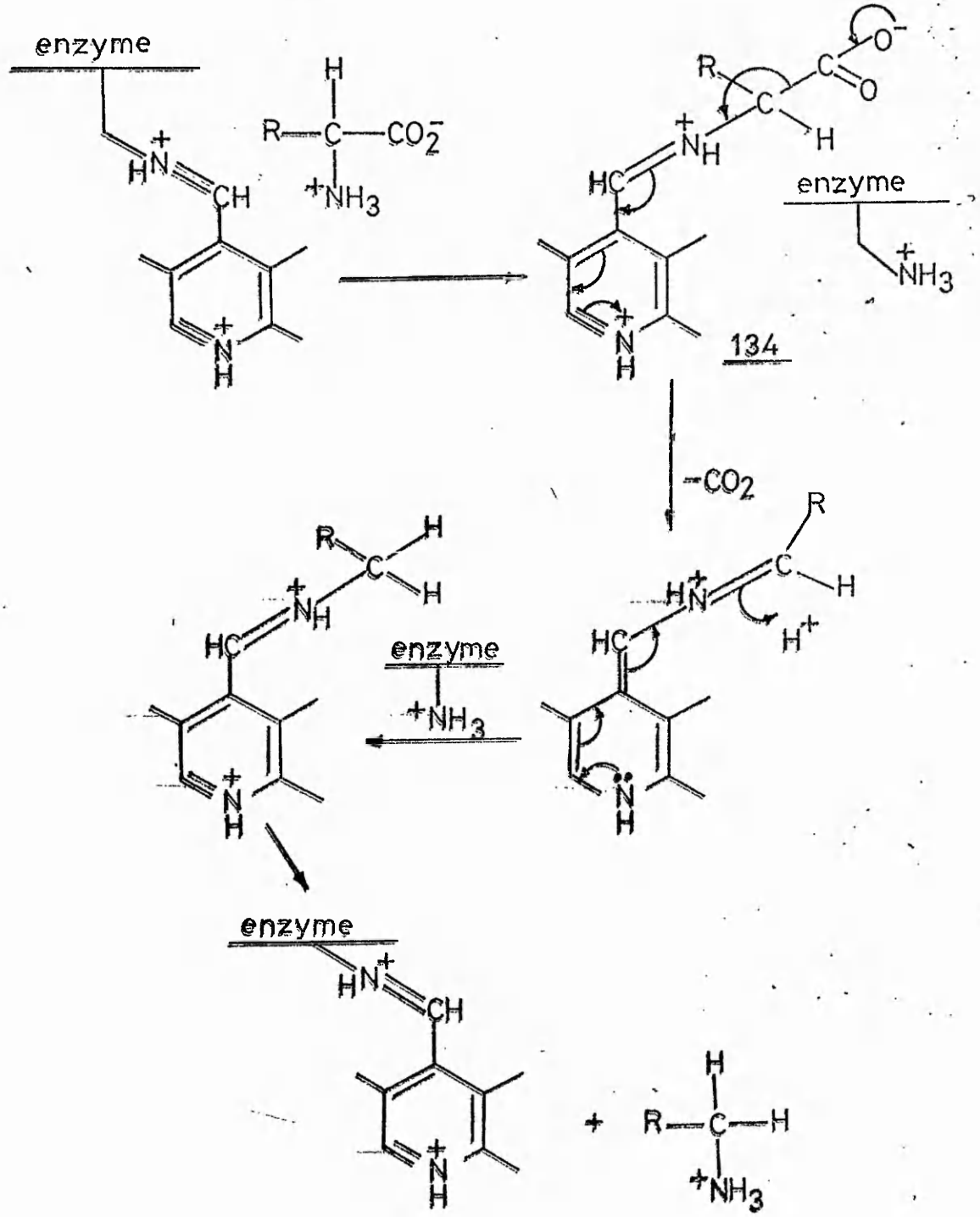
1.3.3 Electrochemical Reactions

Electrochemically induced decarboxylations have long been known and the applications of this reaction to organic synthesis (the Kolbé reaction[152]) has been extensively reviewed.[153-155] The scope of the Kolbé reaction has been widened, to incorporate two related types, the classical Kolbé and the so-called "pseudo" Kolbé reactions, represented by the following equations:



The classical reaction involves dimerisation of two free radical species, whereas a "pseudo" Kolbé reaction involves the further

SCHEME XIV


enzyme
R
C
CO2-
+NH3
H
+N
H
CH
HC
O-
O
enzyme
+NH3
134
-CO2
H+
enzyme
+NH3
enzyme
+N
H
CH
+
R
C
H
+NH3

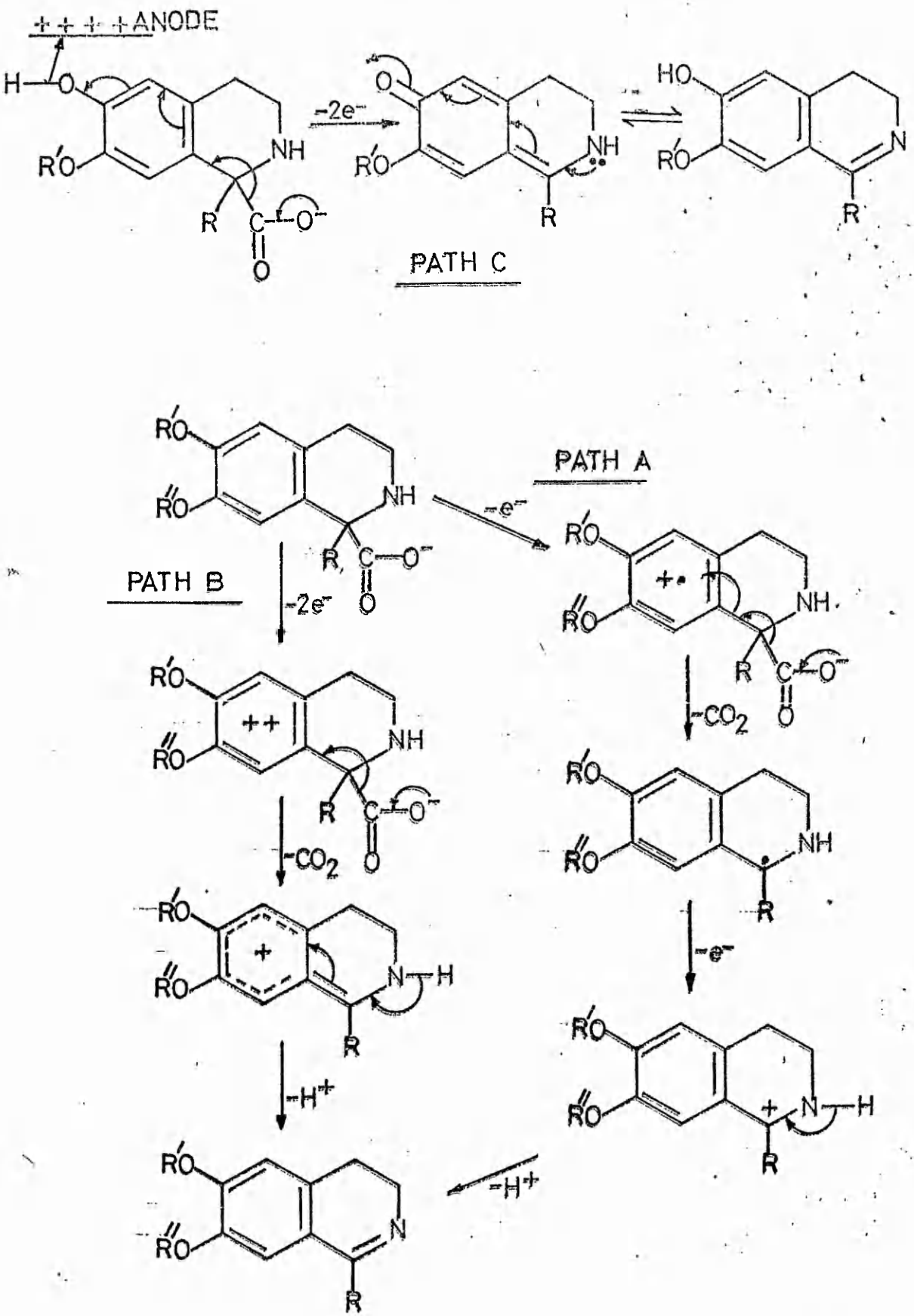
oxidation of the initially formed radical to a carbonium ion. It has been shown[156] that the predominance of one or other of these mechanisms depends (assuming the same experimental conditions are used) upon the ionisation potential of the intermediate radical.

The electrochemical decarboxylation of ∝-amino acids has, in the majority of cases, been accompanied by oxidative degradation of the ensuing amine; e.g. tyrosine anodically decarboxylated at a lead dioxide electrode, gave a complex mixture of products including ammonia, acetic acid, benzoquinone and hydroquinone.[157] Quinuclidine-2-carboxylic acid 136 was considered[158] to be anodically oxidised via a "pseudo" Kolbé pathway, as were the oxidations in methanol of a series of ∝-N-acyl-amino acids, studied by Weedon.[159] An investigation into the anodic decarboxylation of various para-substituted phenylacetic acids, including the para-hydroxy compound, showed[160] that the more electron donating the substituent the greater the extent of carbonium ion formation and the lower the potential required to bring about decarboxylation; e.g. a para-methoxy substituent led to complete "pseudo" Kolbé involvement. The work on anodic oxidation of phenolic isoquinoline-1-carboxylic acids reported by Bobbitt, and discussed earlier (see page 1) could, in one interpretation, be classed as a "pseudo" Kolbé decarboxylation. The very electron-rich aromatic ring undergoes a one-electron oxidation. This is followed by loss of carbon dioxide and an additional one-electron oxidation with subsequent attack by the nitrogen lone pair electrons on the resultant benzyl carbonium ion, i.e. an ECE process, similar to that proposed by Eberson[161] for "pseudo" Kolbé reactions. This and other possible mechanisms for the oxidative decarboxylation of isoquinoline-1-

carboxylic acids are shown in Scheme XV. Path A represents the sequence discussed above. Path C is a concerted two-electron oxidation of a phenol but only applies with a 6-hydroxy function; the significance of this pathway was discussed earlier. The remaining route, path B, is also a concerted two-electron process in which the electrons are taken from the ring at the same time as carbon dioxide is lost. Since there was little difference between the 6- and 7-hydroxyacids in the ease of decarboxylation, path C alone is insufficient to account for the reaction and another mechanism (path A or B), or possibly a combination of such mechanisms, must operate. Decarboxylation of 1-benzylisoquinoline-1-carboxylic acids has also been found[162] to occur in alkaline media under the influence of aerial oxygen, the reaction being more complex than the anodic method, resulting in more than one product being formed. Similarly Wilson and Coscia[163] observed that chemical decarboxylation of 129 gave the 3,4-dihydroisoquinoline 130 to the extent of at least 20%.

1.4 Selection of Suitable Enzymes

Since the intention of part of this project was to study the effect of enzymes upon phenolic isoquinoline-1-carboxylic acids it was necessary to select suitable enzymatic systems. In this respect the phenol oxidases, laccase and tyrosinase, and the peroxidases meet the experimental requirements in that all three catalyse the oxidation of monophenols. Tyrosinases (EC 1.10.3.1), are known to operate by hydroxylation of monophenols to diphenols.[164] The use of these enzymes was therefore judged undesirable in an investigation in which the role of the hydroxylation pattern of the isoquinoline system



SCHEME XV

was to be studied. Although peroxidases have also been known to introduce hydroxyl groups into aromatic systems it was decided to use one of these enzymes (from horseradish root), because of its ready availability in a water-soluble crystalline form.

The following sections discuss the physical and chemical characteristics of both fungal laccase and horseradish peroxidase, and their use in organic synthesis.

1.4.1 Fungal Laccase

Laccase is a member of a group of enzymes known as the blue oxidases.[165] Such copper-containing glycoproteins owe their blue colouration to an intense absorbance near 600nm, identified as an electronic transition of a cysteine sulphur-Cu(II) complex.[166,167] The geometry of this "blue" site has been the subject of much recent work by Gray[168] and other workers.[169]

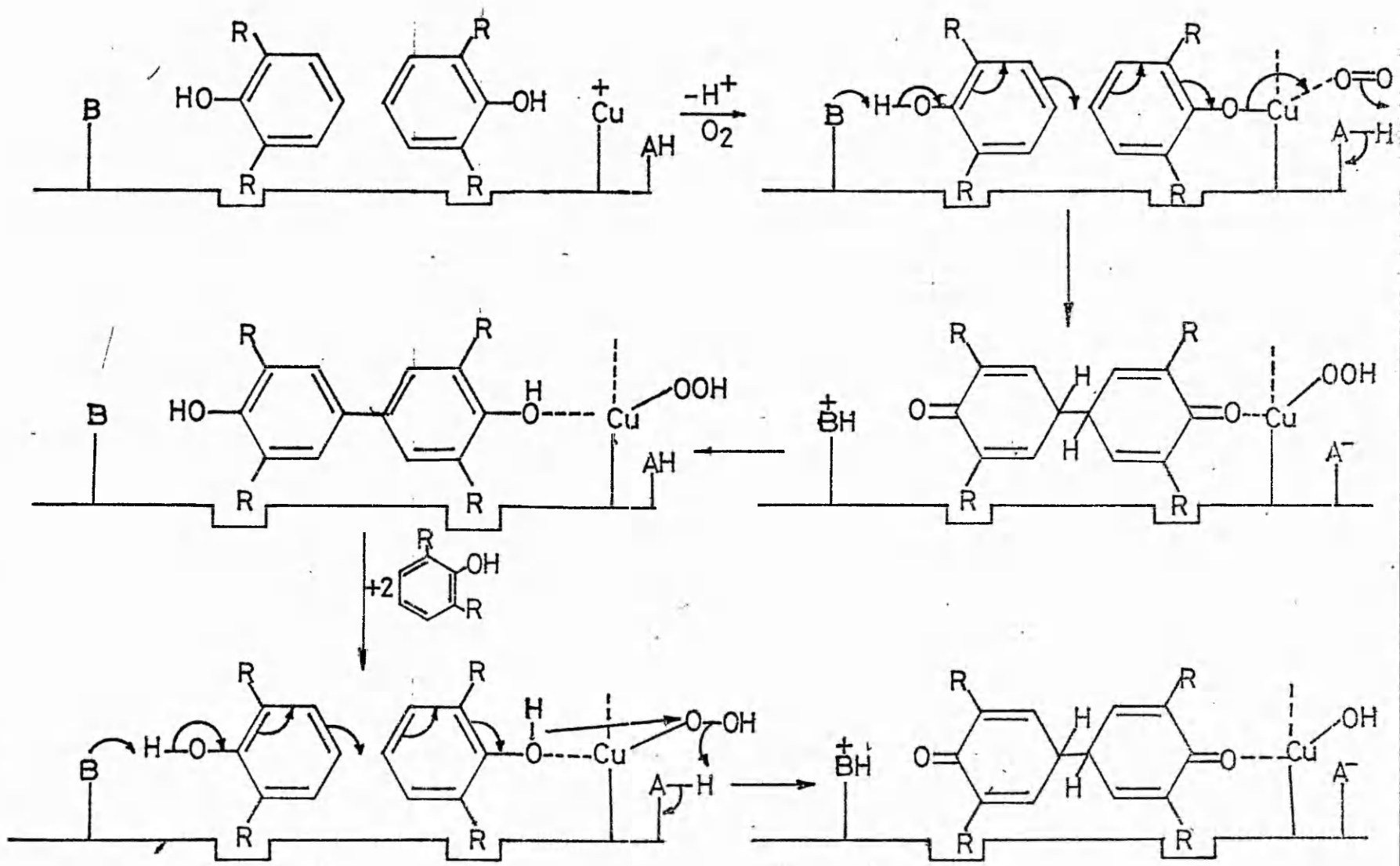
Tree laccase is obtained from the Japanese lac tree (*Rhus vernicifera*[170]), whilst fungal laccase occurs in several fungi mainly Ascomycetes[171] and Basidiomycetes.[172] Fungal laccases are produced extracellularly and it has been suggested that their role is a digestive one, producing nutrients from the rotting wood on which the fungi grow. Most of the work on fungal laccase has involved the enzymes from either *Polyporus versicolor*[173] or *Podospora anserina*.[174] The enzyme from *P. versicolor*, grown in liquid culture, is secreted into the medium and when a suitable inducing agent (e.g. 2,5-dimethylaniline[175]) is added, high yields are possible.

The early literature contains contradictory reports on the substrate specificity of laccase and much work was done to establish whether *o*-, *m*- and *p*-diphenols and *o*-, *m*- and *p*-substituted monophenols, were or

were not oxidised.[176-181] More recent work has been concerned with the mode of action of the enzyme and especially the function and type of copper atoms involved. Fungal laccase contains four copper atoms of which two give EPR signals consistent with Cu(II) ions,[182] one of which has been designated Type 1 and is responsible for the blue colour,[183] the other being designated Type 2 the characteristic of which is the relative ease with which it binds with anionic inhibitors.[184] The two copper atoms, not detectable by EPR are termed Type 3 and were originally thought to be Cu(I) ions to account for their lack of paramagnetism; however more recently it has been suggested[185] that they exist as a pair of linked Cu(II) ions capable of functioning as a two-electron acceptor.[186]

Fungal laccase can accept as many electrons as it has copper atoms,[187] but it has as yet not been established whether more than one electron can be taken at any one time from each substrate molecule. Laccase catalysed oxidations have been shown[188] to produce free radicals, although there is a possibility that such species may arise from side reactions, as demonstrated with tyrosinase.[189] Although the available evidence tends to favour a one-electron oxidation[190] an alternative mechanism in which laccase could effect a two-electron transfer has been proposed,[191] as, for example, in the carbon-carbon coupling of phenols via the intermediacy of a single Cu(II) atom (Scheme XVI). The copper hydroperoxide produced reacts with two additional molecules of phenol, the transfer of the four electrons necessary to reduce an oxygen molecule to two molecules of water being carried out in two two-electron steps.[192]

The uses of fungal laccase in organic synthesis are relatively scarce. Bocks and her co-workers[193,194] have used this enzyme to induce coupling



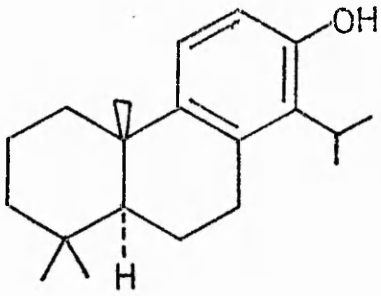
SCHEME XVI

of 2,6-dimethyl- and 2,6-dimethoxyphenol to the corresponding diphenoquinones in 30 and 90% yields respectively. Similarly, enzymic oxidation of pyrogallol gave a quantitative yield of purpurogallin, totarol 137 furnished 20% of podototarin 138 and griseophenone 139 afforded dehydrogriseofulvin 140 in 37% yield.

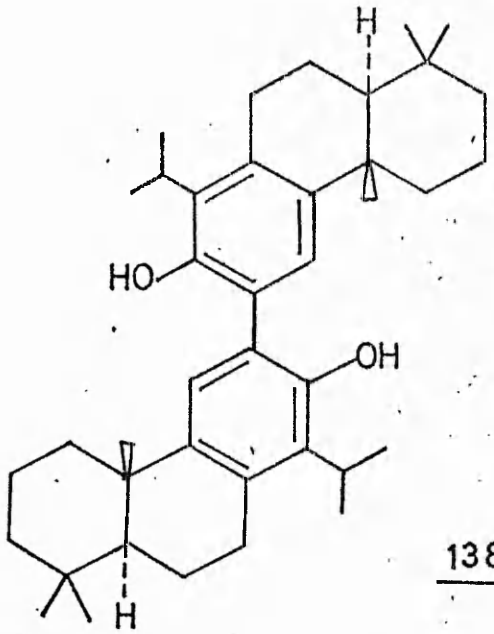
1.4.2 Horseradish Peroxidase

Peroxidases[195] (EC 1.11.1.7) are proteins having the iron-based protoporphyrin IX 141 as the prosthetic group. Peroxidases, found in several animal cells and in virtually all plants, have molecular weights ranging from 30,000 to 100,000. Horseradish peroxidase is the most widely used and studied, being commercially available as a crystalline protein.

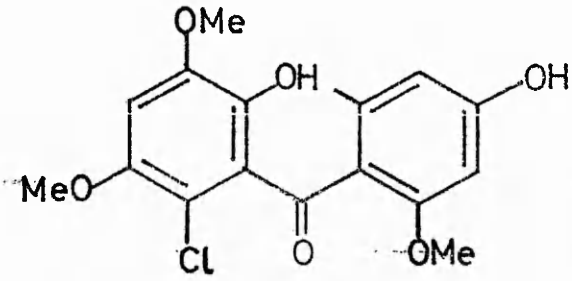
The reaction of the free enzyme with hydrogen peroxide and the nature of the products formed have been extensively investigated. Early work[196,197] showed that the initial green product (designated HRP-I) could, by a subsequent one electron reduction, yield a red product (HRP-II), which after a further one-electron reduction reformed the free enzyme. The nature of HRP-I and II was originally thought to be that of enzyme-substrate complexes, until it was found that HRP-I was not in equilibrium with the free enzyme. Furthermore HRP-I could be produced by the action of other oxidants (e.g. molybdicyanide[198]). The enzyme therefore reacts with hydrogen peroxide to give genuine compounds and additional work[198] has characterised two more intermediates, HRP-III and ferroperoxidase. In horseradish peroxidase the iron atom is in the Fe(III) oxidation state while in ferroperoxidase it exists as an Fe(II) atom. The relationship[199] between these compounds is illustrated in Scheme XVII; the numbers in parentheses refer to the "oxidising



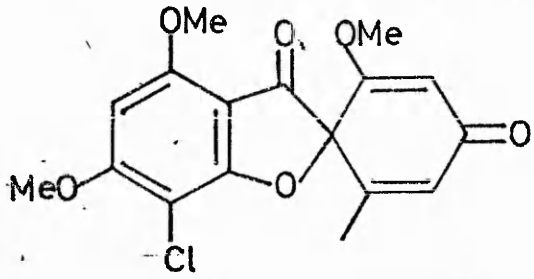
137



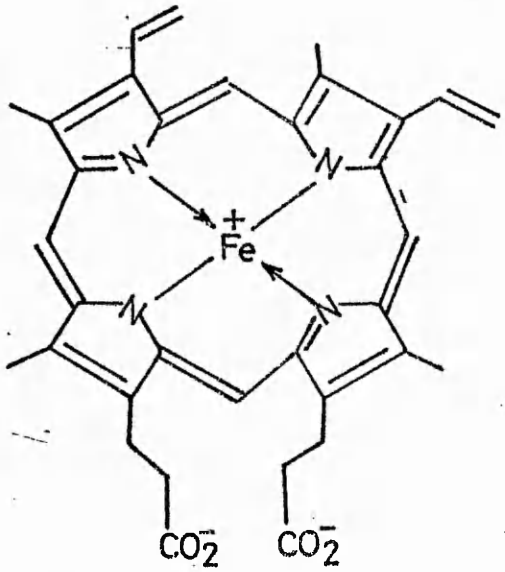
138



139



140



141

equivalent" and not necessarily to the oxidation state of the iron atom. Similar compounds can be formed between the enzyme and the simple alkyl hydroperoxides.

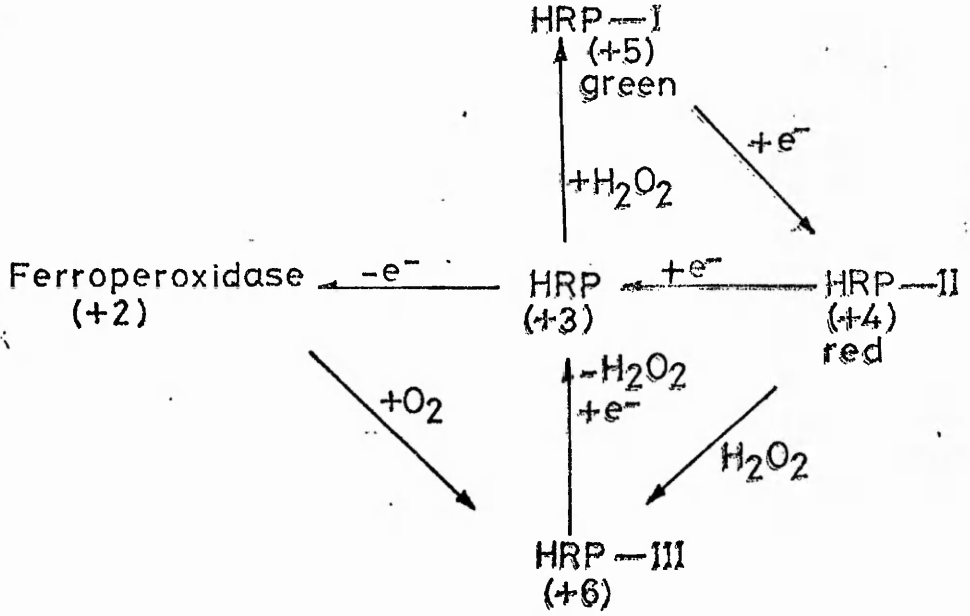
The iron atom bound in the porphyrin ring has six co-ordination sites, one being to the protein residue and four with the pyrrole nitrogens. It has been suggested[200] that in HRP-I an oxygen anion is positioned at the remaining co-ordination site with the iron atom being in the Fe(V) state, and when it is reduced to HRP-II the iron is in the ferryl state (FeO^{2+}).[201] Another theory, proposes[202,203] that the porphyrin ring is oxidised by peroxide in HRP-I and that reduction to HRP-II converts the iron from the ferric to ferrous state. No substantial evidence for either of the above two theories has been produced.

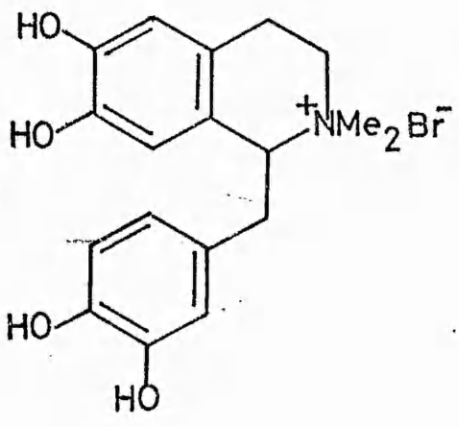
The reaction of HRP compounds with hydrogen donors is thought[204] to involve the generation of free radicals as follows:—

$$\text{HRP} + H_2O_2 \longrightarrow \text{HRP-I}$$
$$\text{HRP-I} + AH_2 \longrightarrow \text{HRP-II} + AH\cdot$$
$$\text{HRP-II} + AH_2 \longrightarrow \text{HRP} + AH\cdot$$
$$2AH\cdot \longrightarrow \text{products}$$

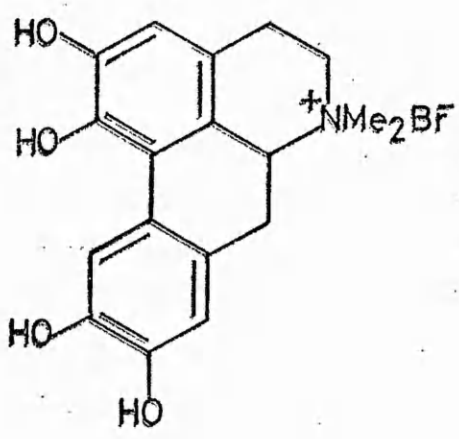
The important consequence of this mechanism is that if the HRP compounds do not bind to the hydrogen donor, then the reaction will be bi-molecular and will not show saturation kinetics characteristic of enzyme reactions. Such kinetics were found[205] to apply with the reaction of HRP-II and ferrocytochrome c. A more recent investigation[206] studied the reaction of HRP-I and HRP-II with iodide, ferrocyanide, 4-cresol and 4-aminobenzoic acid; the results suggest that neither iodide nor ferrocyanide formed enzyme-substrate complexes, whereas the latter two almost certainly involved the formation of such species.

SCHEME XVII





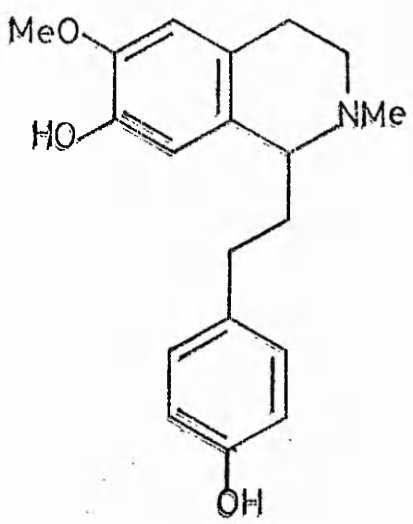
142



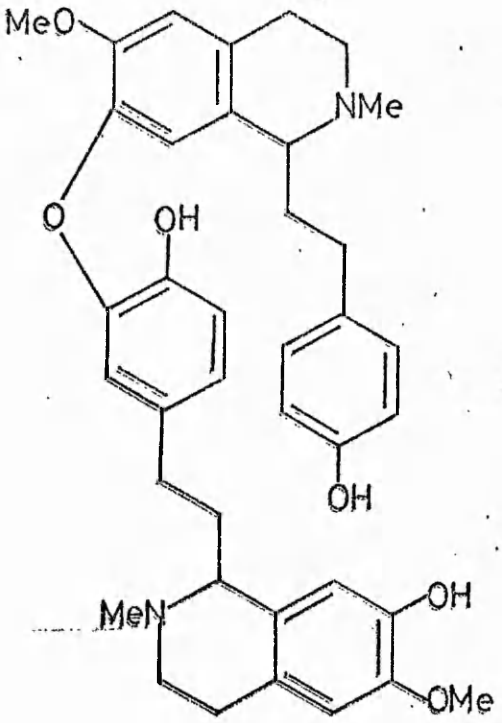
143

The biological role of peroxidase is uncertain[207] although it is known that a wide range of compounds, including aromatic amines, phenols, enediols, leuco dyes and amino acids, can act as hydrogen donors to horseradish peroxidase. The many suggestions which have been made as to the function of peroxidase include the catalysis of phenol oxidative coupling (e.g. in the biosynthesis of lignin[209]), the removal of hydrogen peroxide from the cell,[207] the hydroxylation of aromatic compounds[210] and the decarboxylation of amino acids.[211]

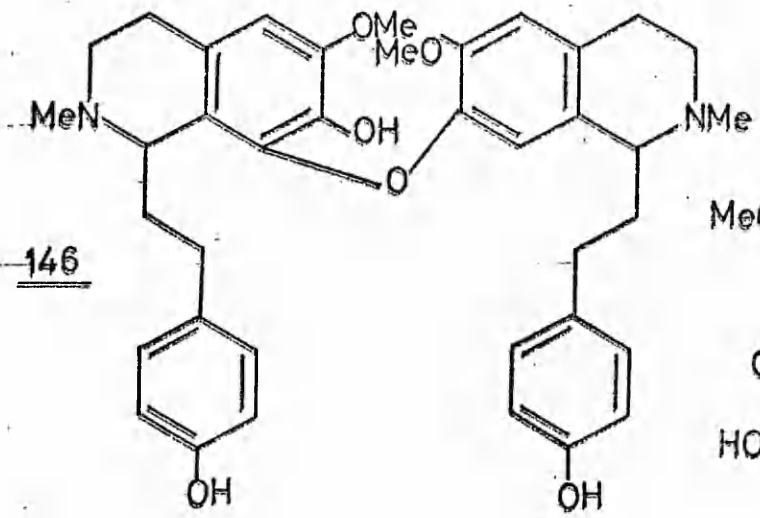
In contrast to laccase, peroxidase has been widely used in the field of organic synthesis and therefore only those reactions applicable to isoquinoline alkaloids will be discussed. The oxidation of laudanosoline methobromide 142 by the enzymes, mushroom tyrosinase, Rhus laccase and horseradish peroxidase has been investigated;[212] chromatographic and spectral evidence for the formation of the quaternary aporphine 143 was obtained. Kametani and his co-workers have used mixtures of crude plant homogenates and hydrogen peroxide as oxidants. Oxidation of the phenylethylisoquinoline 144 with potato peel and peroxide gave[213] the head-to-tail dimer 145 in low yield and a Wasabia japonica homogenate afforded[214] the head-to-head dimer 146 in 25% yield. The authors proposed that different enzymes, specific for each type of coupling were present in the plants. Oxidation of N-methylcoclaurine by the potato peel system was reported[215] to give very small yields of the dimer 147 and a similar trimer, but only mass spectroscopic evidence was adduced for the structures of the products. An attempt[216] to bring about coupling of reticuline 51 to salutaridine 94 with an homogenate of Papaver rhoeas (a plant which does not contain morphine alkaloids) and peroxide, led only to hydroxylation of reticuline at the β-position. The attempted oxidation of N-methylcoclaurine and its 7-O-benzyl ether



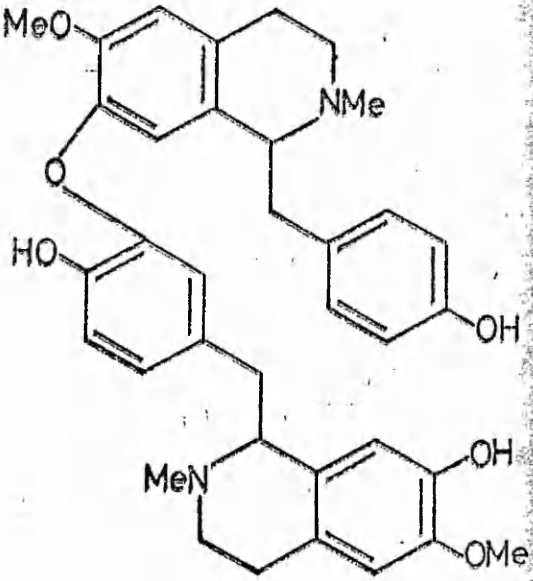
144



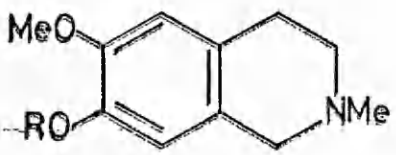
145



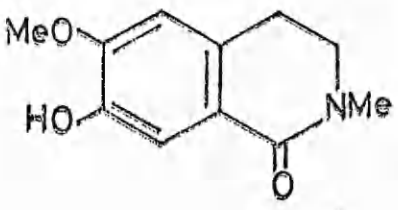
146



147

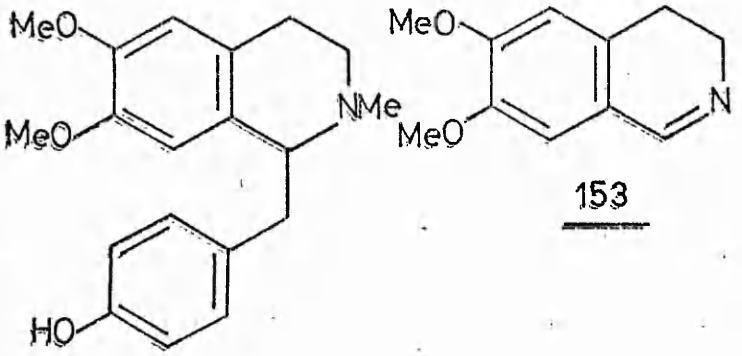


148 R=H
149 R=PhCH$_2$O
152 R=Me



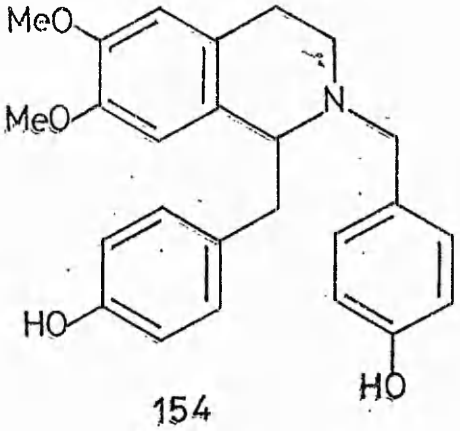
150

with pure horseradish peroxidase and peroxide resulted[217] in cleavage of the molecule with loss of the 4-hydroxy-benzyl grouping to give the corypallines 148 and 149 respectively. Similarly reticuline gave the cleaved product thalifoline 150. Peroxidase oxidation[218] of armepavine 151 produced O-methyl corypalline 152, while N-norarmepavine yielded the dihydroisoquinoline 153 and the 1,2-dibenzylisoquinoline 154 (formed by addition of the 1,4-benzoquinone methide, produced in the oxidative cleavage, to the parent isoquinoline). Oxidative cleavage of this type was also observed when 4'-hydroxybenzylisoquinolines were oxidised anodically.[219] N-methylisosalsolidine was converted[218] by horseradish peroxidase and peroxide into a mixture of the carbon-carbon and carbon-oxygen-carbon dimers 155 and 156, while lophocerine 157 afforded the dimer 158 in low yield. Similar treatment of N-methyl-coclaurine gave the diphenyl ether 159, along with two dihydroisoquinoline products. The horseradish peroxidase-peroxide oxidation of the aporphine alkaloid boldine, produced[220] both the carbon-carbon and carbon-oxygen-carbon dimers 160 and 161 in small amounts. Similar oxidations of quaternary isoquinolines has been found to give substantially better yields of coupled products. Laudanosoline hydrobromide afforded[221] in 81% yield the dibenzopyrrocoline 162, whilst the methobromide analogue gave[220] the aporphine 143 in 60% yield, supporting the preliminary results of Frömming.[212] The improvement in the reaction obtained by quaternisation of the nitrogen is in accord with the observations of Franck and his collaborators.[222] The most likely explanation is that removal of the lone pair electrons prevents cleavage of the 1-benzyl group.

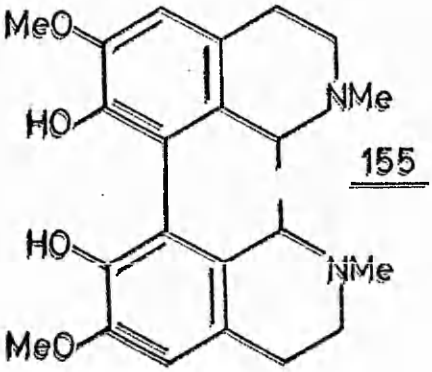


151

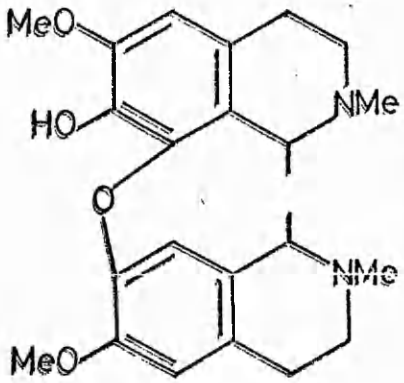
153



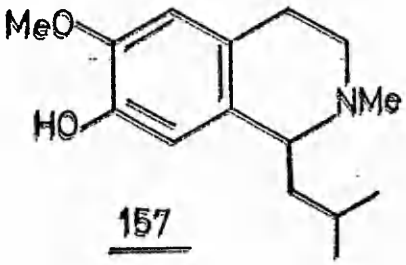
154



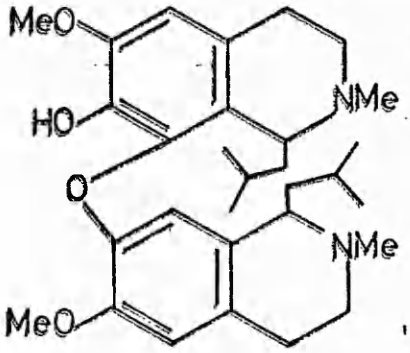
155



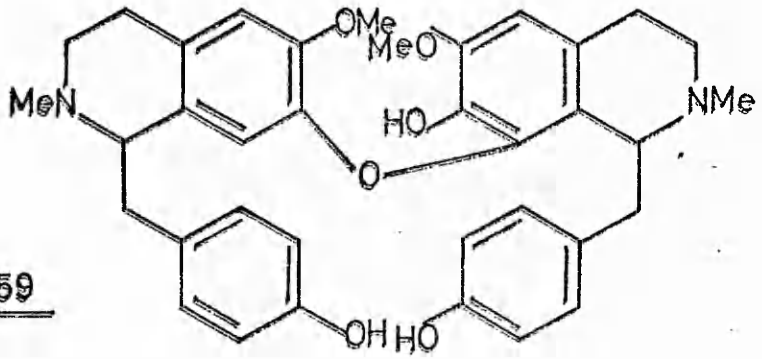
156



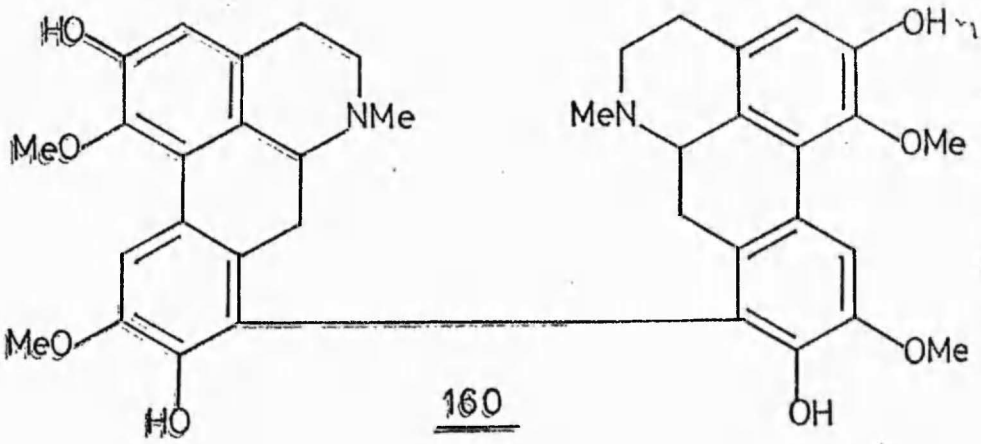
157



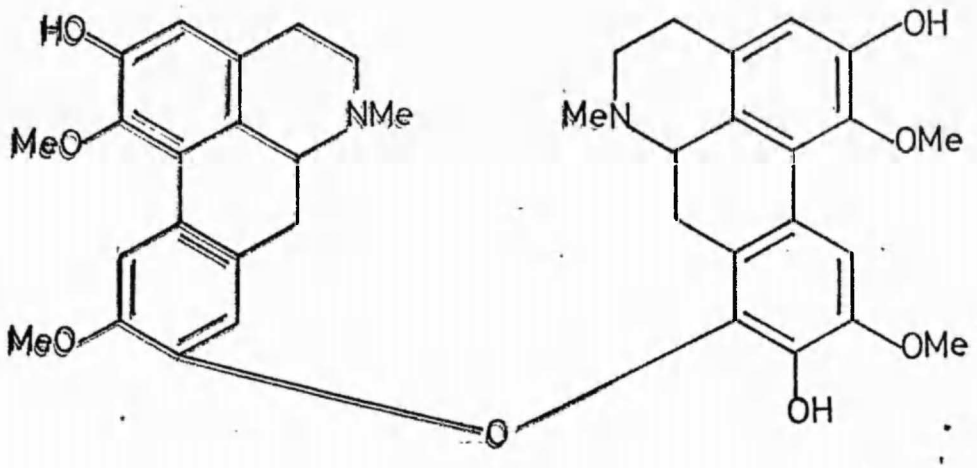
158



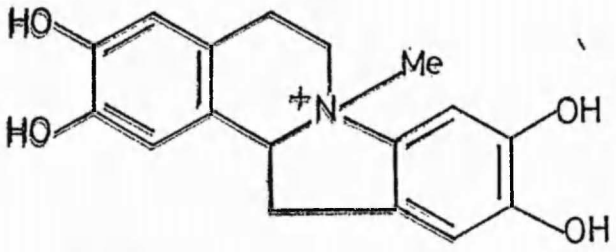
159


HO
MeO
NMe
MeO
HO
MeN
OH
OMe
OMe
OH

160


HO
MeO
NMe
MeO
MeN
OH
OMe
OMe
OH
O

161


HO
HO
Me
+N
OH
OH

162

CHAPTER TWO

THE ENZYME-CATALYSED DECARBOXYLATION OF ISOQUINOLINE-1-CARBOXYLIC ACIDS

Bobbitt[1] has recently reported that a series of 1-benzyl isoquinoline-1-carboxylic acids possessing either a 6- or 7-hydroxy function in the isoquinoline nucleus, can readily be oxidatively decarboxylated to the corresponding 3,4-dihydroisoquinolines on a graphite electrode at low potentials. As a result of this reaction, it was proposed that in certain cases electrochemical reactions could mimic *in vivo* reactions. A study was therefore undertaken into the effect of enzymic oxidants on phenolic isoquinoline-1-carboxylic acids. The enzymes selected for this purpose were fungal laccase and horseradish peroxide for the reasons discussed in Section 1.4.

2.1 Preparation of Enzymes

The Basidiomycete, *Polyporus versicolor* was grown in static cultures of the liquid medium described by Fahreus and Reinhammer.[175] The formation of the extracellular enzyme was induced after seven days growth of the fungus by the introduction of 2,5-dimethylaniline. The cultures were harvested after an additional seven days growth and were stored at -20° until required. Assay of the crude enzyme preparation was conducted according to the method of Pickard and Westlake,[223] using catechol as substrate. The activity of the crude enzyme solution was in general between 1 and 4 units/cm^3 (for definition of the unit, see Experimental). Attempts to purify the laccase preparation by an ammonium sulphate precipitation technique led to the loss of so much activity that it was decided to use the crude culture filtrate.

Horseradish peroxidase is commercially available as the water soluble crystalline solid; its activity was determined according to the method

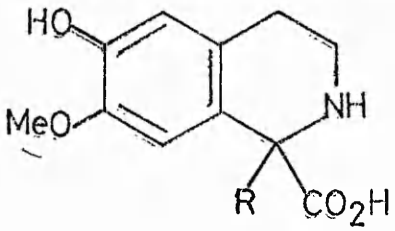
described by Devlin.[224]

2.2 Preparation of Substrates

For the enzyme decarboxylation studies it was necessary to prepare three different types of 1,2,3,4-tetrahydroisoquinoline-1-carboxylic acids, having different substitution patterns in the isoquinoline nucleus, viz. 6-hydroxy-7-methoxy, 6-methoxy-7-hydroxy and 6,7-dimethoxy. In addition, it was also desired to prepare acids having various groups at the C-1 position; hydrogen, methyl, benzyl and 3,4,5-trimethoxybenzyl were chosen as substituents, sufficiently different, to enable any trends within each series to be examined. Synthesis of the 3,4-dihydroisoquinolines, expected to arise from the decarboxylations, was also necessary for assay purposes.

The 3-benzyloxy-4-methoxy-, 3-methoxy-4-benzyloxy-, and 3-hydroxy-4-methoxy-β-phenylethylamines, required for the synthesis of the phenolic acids and 3,4-dihydroisoquinolines, were prepared by well established procedures.[225-7]

3,4-Dimethoxy-β-phenylethylamine (homoveratrylamine) is commercially available and was used without purification. 6-Hydroxyacids were prepared by Pictet-Spengler condensations[136] of the 3-hydroxy-4-methoxy-β-phenylethylamine hydrochloride with the appropriate α-keto acids. The novel 1-hydrogen acid 163 was synthesised in 47% yield according to the method of Harvey et al,[229] using glyoxylic acid in 0.05M sulphuric acid, whereas the known acid 164,[2,230] and the novel acids 165 and 166 were prepared by condensation of the amine and pyruvic acid under physiological conditions (i.e. 25° and pH 4.5-6). With the exception of 3,4,5-trimethoxyphenylpyruvic acid 167, the α-keto acids were

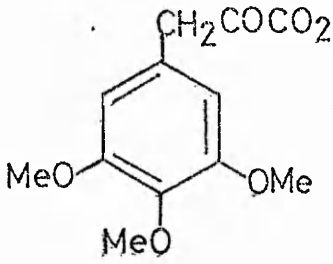


163 R=H

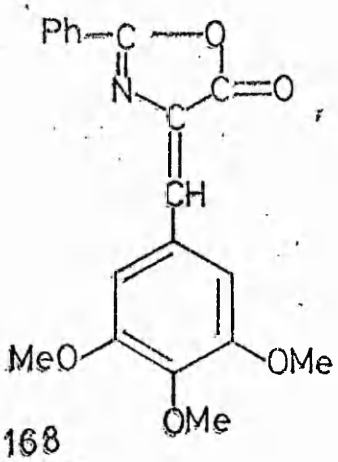
164 R=Me

165 R=$PhCH_2$

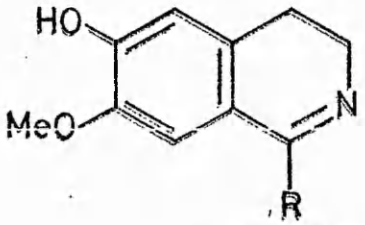
166 R=3,4,5$(MeO)_3PhCH_2$



167



168

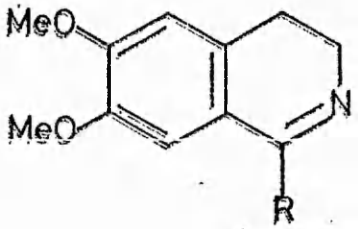


169 R=H

170 R=Me

171 R=$PhCH_2$

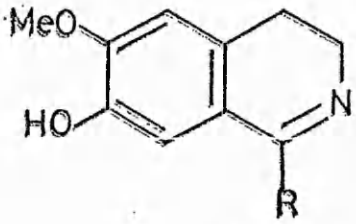
172 R=3,4,5$(MeO)_3PhCH_2$



173 R=H

174 R=Me

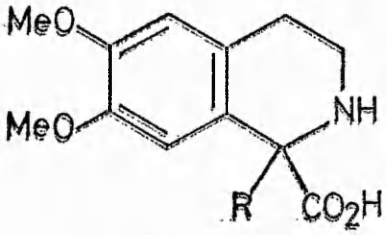
175 R=$PhCH_2$



176 R=H

177 R=Me

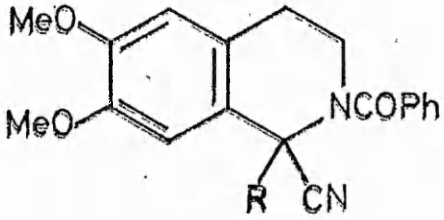
178 R=$PhCH_2$



179 R=H

180 R=Me

181 R=$PhCH_2$



182 R=H

183 R=Me

184 R=$PhCH_2$

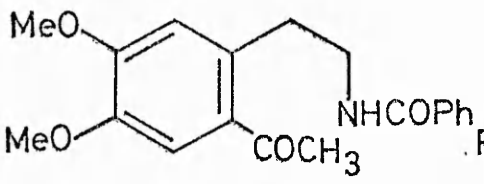
commercially available. Preparation of 167 was achieved in 56% yield overall from the substituted benzaldehyde, by base hydrolysis[231,232] of the derived azlactone 168. The isoquinoline-1-carboxylic acids were converted into their hydrochloride salts to aid their solubility in water.

The 3,4-dihydroisoquinolines 169, 170, 171 and 172 were obtained in moderate yields from Bischler-Napieralski cyclisations[228] of the corresponding N-acyl-3-benzyloxy-4-methoxy-β-phenylethylamines using phosphorus oxychloride, followed by O-debenzylation with 1:1 ethanol-conc, hydrochloric acid. 3,4,5-Trimethoxyphenylacetic acid, required for the preparation of 172 was obtained from the corresponding pyruvic acid 167 according to the method described by Snyder et al.[233] Similarly the 6,7-dimethoxy 173, 174 and 175 and the 6-methoxy-7-hydroxy-3,4-dihydroisoquinolines 176, 177, 178 were made from homoveratrylamine and 6-methoxy-7-benzyloxy-β-phenylethylamine respectively, via the appropriate N-formyl, N-acetyl and N-benzoyl derivatives.

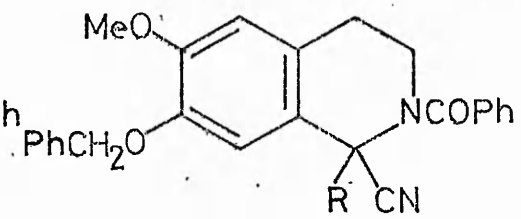
Synthesis of the 6,7-dimethoxy acids 180 and 181 involved the initial preparation of the dihydro-Reissert compounds[234] 182, 183 and 184. The now widely employed two phase method for making Reissert compounds, developed by Popp,[235] was used to prepare 182 in 54% yield. Subsequent alkylation, using sodium hydride to generate the Reissert anion, followed by electrophilic addition of the required alkyl halide, gave the 1-alkyl Reissert compounds 183 and 184. An attempt to make 183 directly from the 1-methyl-3,4-dihydroisoquinoline 174 according to the above method, resulted in only 8% of the desired product being obtained. The major product was the compound 185, presumably arising from hydrolysis of

the acylated Schiff's base. Its identity was confirmed by comparison with an authentic sample, prepared by the method of Forbes et al.[236]

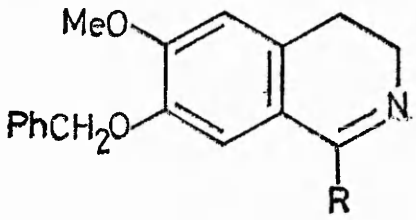
Reaction of Reissert compounds with strong acids has long been known to cause hydrolysis of the amide and nitrile functions; treatment of the dihydro-Reisserts 183 and 184 with 85% phosphoric acid[234] gave the dimethoxy acids 180 and 181 in 51% and 74% respectively. The 1-hydrogen acid 179 was synthesised in an identical manner by Dr M.R. Hamblin of this laboratory. The planned route to the 7-hydroxy acids also involved preparation and hydrolysis of dihydro-Reissert products. Reissert compound 186 was prepared in 46% yield from the 3,4-dihydroisoquinoline 189 by the two phase method. A minor product from the reaction was assigned the structure 191 from spectral data and by analogy with the formation of 185, although elemental analyses were not in good agreement. Methylation of the dihydro-Reissert anion from 186 afforded N-benzoyl-1-cyano-1-methyl-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 187 in 80% yield. It was also found that the attempted synthesis of 187 directly from the 1-methyl-3,4-dihydroisoquinoline 190 gave the ring cleaved compound N-benzoyl-(2-acetyl-4-benzyloxy-5-methoxy-β-phenyl)-ethylamine 192, as the sole product. Similar reaction of the anion from 186 with benzyl chloride gave the novel 1-benzyl Reissert compound 188 in 67% yield. It has been reported[1] that as well as modifying the N-benzoyl and nitrile functions, hydrolysis with phosphoric acid removes O-benzyl groups, affording phenolic products. Unfortunately numerous attempted hydrolyses, by the author and also by Dr M R Hamblin, of the 7-benzyloxy Reisserts 186, 187 and 188 with 85% phosphoric acid failed to give any of the expected 7-hydroxy acids as did attempted hydrolyses with hydrochloric acid and with less strong solutions of phosphoric acid. Other possible



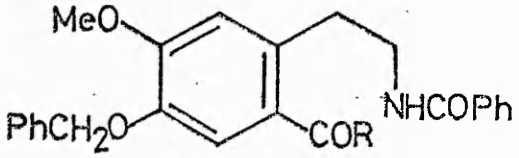
185



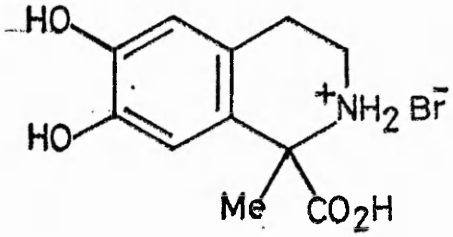
186 R=H
187 R=Me
188 R=PhCH$_2$



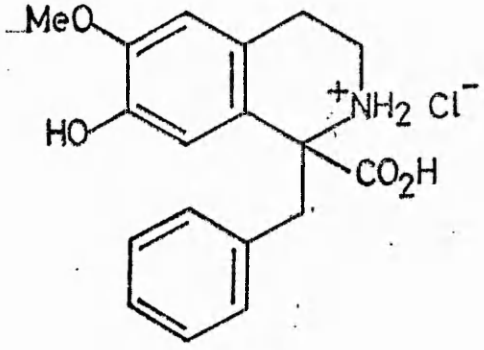
189 R=H
190 R=Me
353 R=PhCH$_2$



191 R=H
192 R=Me



193



194

routes to such acids involving partial hydrolysis of the Reissert nitrile group with sodium hydroxide solution[238] or Amberlite IR-4B-(OH) ion exchange resin[239] were also unsuccessful, as was reduction with lithium aluminium triethoxyhydride.[240] The attempted partial demethylation of dimethoxy acid 180 at the C-7 position using hydrobromic acid[241] was also unsuccessful giving 193 as the sole product. A sample of 1-benzyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 194 was obtained from Prof. J.M. Bobbitt.

2.3 The Enzyme-Catalysed Decarboxylation

Since laccase acts primarily on phenolic systems, it was not expected that the 6,7-dimethoxyacids 179, 180, and 181 would undergo decarboxylation, and they were employed simply to check for the presence of any other decarboxylating or hydroxylating enzymes in the crude laccase preparation. Initial studies were carried out in collaboration with Dr M R Hamblin and involved dissolving the carboxylic acids in phosphate buffer (pH 6.0) with incubation for 72 hr. Additional amounts of enzyme were introduced after 24 and 48 hr. The reaction mixtures were made alkaline by the addition of ammonia solution, and continuously extracted with chloroform. Recovery of the reaction products was poor; e.g. the 6-hydroxy acid 164 afforded after incubation and extraction only 18% of a brown product, shown by its t.l.c. behaviour and picrate derivative[242] to be identical with the dihydroisoquinoline 170. The dimethoxy acids, after incubation with laccase and subsequent basification, did not give any chloroform soluble material, neither did the reaction mixtures exhibit the characteristic blue fluorescence of dihydroisoquinoline solutions.

When the dihydroisoquinoline 170 was dissolved in the same buffer

system, basified and continuously extracted with chloroform, the recovery was only 30%, suggesting that isolation of the water-soluble products by extraction into organic solvent was not an ideal way of following the decarboxylation reactions.

The possibility of following the reaction by u.v. spectrophotometry was then investigated. Phenolic-3,4-dihydroisoquinolines are reported[243] to show strong absorption maxima above 340 nm, and this was found to be the case with all the isoquinolines studied. These maxima occur in a region in which the isoquinoline-1-carboxylic acids do not absorb, and are thus a convenient method of assaying the formation of dihydroisoquinoline compounds. The absorption maxima for the 3,4-dihydroisoquinoline compounds along with their extinction coefficients are presented in Table I.

TABLE I

Isoquinoline	Wavelength nm	ε
169	389	12870
170	375	7883
171	389	10947
172	392	17212
173	354	6263
174	346	8528
175	352	8299
178	355	6550

Solutions of all the isoquinoline-1-carboxylic acids in phosphate buffer (pH 6.0) were found to be remarkably stable, u.v. measurements showing less than 5% dihydroisoquinoline content after standing for 24 hr. Similarly no decarboxylation occurred on standing with either peroxidase solution or peroxide solution.

Next, the rates of the laccase- and horseradish peroxidase-induced decarboxylations of the prepared isoquinoline-1-carboxylic acids were studied. The reaction was carried out using calibrated amounts of acid, enzyme (peroxide also in the case of the peroxidase studies), and buffer in a 1cm silica cell. Any increase in absorbance with time at a specific wavelength was electronically recorded, and by drawing tangents to the resulting curves from a number of different substrate concentrations, the initial velocity of the reaction was calculated. The selected wavelengths were those presented in Table 1. The actual method used to carry out each run is described in the Experimental section. None of the 6,7-dimethoxyacids 179, 180 or 181 showed any increase in absorbance when incubated with either laccase or horseradish peroxidase-peroxide for up to 2 hr.

In general the initial velocity of an enzyme reaction is given[244a] by the Michaelis-Menten equation,

$$v = \frac{V}{1+K_s/s}$$

where v is the initial velocity, V is the maximum velocity, found when the enzyme is saturated with substrate, K_s is the equilibrium constant for the reaction $ES \rightleftharpoons E+S$, i.e. the dissociation constant of the enzyme-substrate complex, and s is the substrate concentration. When s is equal to K_s, the initial velocity is half of the maximum velocity, and this value of s is termed K_m, the Michaelis constant. The above equation can also be expressed as:

$$\frac{1}{v} = \frac{K_m}{V} \times \frac{1}{s} + \frac{1}{V}$$

thus a plot of $\frac{1}{v}$ against $\frac{1}{s}$ will give a straight line which will intercept the substrate concentration axis at a point giving $-1/K_m$ and the

velocity axis at a point giving $\frac{1}{V}$. This is the Lineweaver-Burk[245] method of determining K_m and V, and is probably the one most widely used. The above treatment holds for the vast majority of enzymes, but there is doubt about its applicability to peroxidase, which is reported not to form substrate-enzyme complexes (see Section 1.4.2). Nevertheless, runs carried out with peroxidase did show saturation kinetics and reproducible values of K_m and V were obtained. The data given in Appendix I were used to prepare Lineweaver-Burk plots and the constants K_m and V were determined for each reaction; the calculated values are presented in Table II.

TABLE II

Acid	K_m(Run 1) $[10^{-4}M]$	K_m(Run 2)	V(Run 1) $[10^{-7}$ mol $min^{-1}]$	V(Run 2)
(a) Laccase				
163	0.4	0.4	0.2	0.2
164	1.3	1.2	0.5	0.6
165	14.0	10.0	10.0	6.7
166	2.0	3.0	3.3	4.0
194	3.7	-	0.2	-
(b) Peroxidase				
163	1.1	1.2	0.6	0.5
164	2.5	1.7	2.5	1.7
*165	20.0	20.0	17.0	20.0
165	20.0	-	50.0	-
166	12.5	10.0	14.0	10.0
194	0.9	-	0.04	-

*These two runs were conducted using exactly half the quantity of enzyme employed in the other peroxidase experiments.

It is apparent that the value of K_m is independent of the enzyme concentration, whilst the value of V is directly proportional to it; all of the laccase runs were carried out with identical amounts of the same enzyme solution, and the values of V are therefore directly comparable. The same applies to peroxidase except as mentioned in the footnote to Table II.

The determination of K_m and V provides information about both parts of an enzyme reaction, i.e. the formation of the enzyme-substrate complex and the subsequent breakdown of this complex to give the reaction products. The magnitude of K_m is inversely proportional to the affinity of the enzyme for the substrate, while the value of V is a measure of the speed at which the complex breaks down.[244b] Prolonged incubation of the 6-hydroxy acids with laccase and peroxidase showed an increase in absorption at the selected wavelength, which after reaching a maximum then decreased.

The inference is that the initial product, i.e. the 3,4-dihydroisoquinoline, was being further oxidised. This was confirmed by incubating with both enzymes, the phenolic 3,4-dihydroisoquinolines 169, 170, 171 and 172, all of which exhibited a decrease in absorbance at the appropriate wavelength. The occurrence of a competing reaction renders the interpretation of the kinetic data more debatable. However, since the experimental readings were taken during the initial stages of each reaction, i.e. when the concentration of dihydroisoquinoline was small and therefore the effect of the competing reaction was at a minimum, it was considered that the results presented in Table II were a reasonable reflection of the actual kinetics. None of the 6,7-dimethoxy-3,4-dihydroisoquinolines was affected by either enzyme. With the reservation that there is a competing reaction, the following trends are evident from the results of the kinetic study:-

(i) both laccase and horseradish peroxidase are able to catalyse the oxidative decarboxylation of both 6- and 7-hydroxyisoquinoline-1-carboxylic acids, albeit to differing extents, whereas neither was effective on the 6,7-dimethoxy analogues;

(ii) with laccase, the 1-benzyl-6-hydroxy acid 165 produces significantly larger values of K_m and V, i.e. the subsequent break-down of the enzyme-acid complex is substantially faster than for other 6-hydroxy acids;

(iii) peroxidase shows a similar specificity for the two 6-hydroxy acids 165 and 166 possessing a 1-benzyl residue, resulting in K_m and V values at least an order of magnitude higher than for the other two 6-hydroxy acids;

(iv) a marked difference is apparent in both the formation of the acid-enzyme complex and the rate at which it is broken down, between the 1-benzyl-6- and 7-hydroxy acids 165 and 194 using laccase and peroxidase, with the former compound being the faster;

(v) although with laccase it was found that 194 gave K_m and V results comparable to those from 6-hydroxyacids (except the 1-benzyl), it would appear that with peroxidase the acid-enzyme complex is significantly slower in breaking down.

The greater degree of specificity shown by peroxidase is surprising, considering the reported non-formation of enzyme-substrate complexes, and free-radical mechanism (see Section 1.4.2).

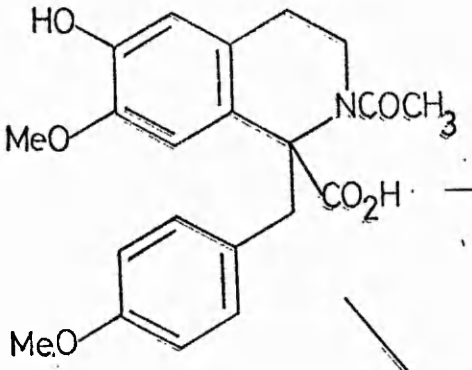
It has, then, been established that phenolic tetrahydroisoquinoline-1-carboxylic acids are oxidatively decarboxylated by enzymes which occur in plant cells, with the results being broadly parallel to those found in the anodic decarboxylation study, except for the marked difference between the 1-benzyl acids. In order for more accurate kinetic parameters to be derived from such enzyme-catalysed decarboxylation reactions, further work is necessary to identify and assess the extent to which the competing reaction occurs.

CHAPTER THREE

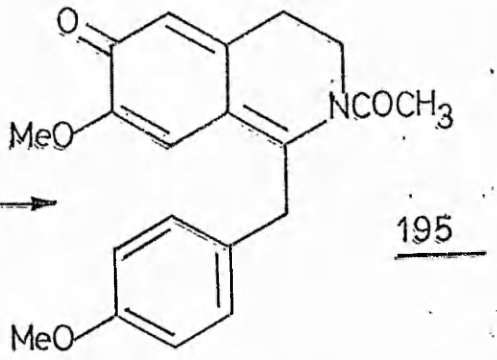
Attempted Synthesis of an Aporphine Alkaloid by Oxidative Decarboxylation of an Isoquinoline-1-Carboxylic Acid

Of potential importance in the area of isoquinoline alkaloid biosynthesis is the isolation[1] of the quinonoidal compound 195 and its tautomer 196 in 15% and 9% yield respectively, from the anodic decarboxylation of the N-acetylated isoquinoline-1-carboxylic acid 197. The generation of such quinone methide compounds with subsequent non-oxidative coupling by either intra- or intermolecular processes could possibly give rise to a range of tetracyclic and bisbenzylisoquinoline alkaloids. To the former category belongs the aporphine class of alkaloids, and the aim of the work described in this chapter was to investigate the possible synthesis of an aporphine alkaloid by the electrochemical, chemical and enzymatic oxidation of N-protected isoquinoline-1-carboxylic acids. The N-acyl-1-(3′,4′,5′-trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 198 was selected as the system to be studied since the polymethoxylated 1-benzyl residue was considered to be sufficiently nucleophilic to bring about intramolecular attack on the postulated quinone methide part of the oxidised molecule, and being symmetrical, would in such an event, give rise to only one product, irrespective of whether coupling took place at the C-2′ or C-6′ position.

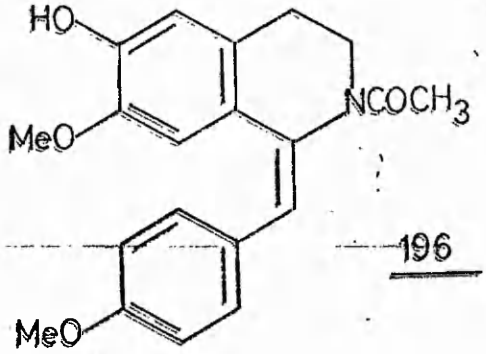
A possible mechanism for the formation of the appropriately substituted aporphine-type compound by oxidative decarboxylation of the phenolic acid 198, *via* a quinone intermediate and intramolecular cyclisation is shown in Scheme XVIII. The expected product from such a route is the 6a, 7-dehydroaporphine 199. It was thought necessary, in anticipation of low yields, to synthesise a substituted dehydroaporphine (either 199 or 200) to facilitate the identification of such a product from



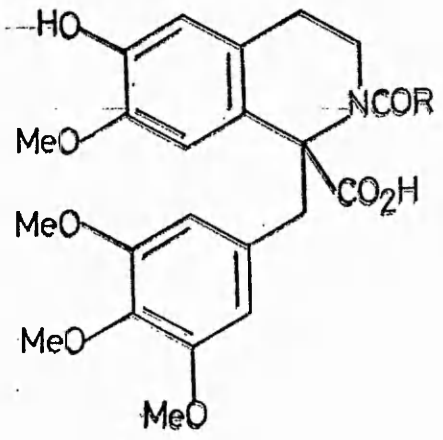
197



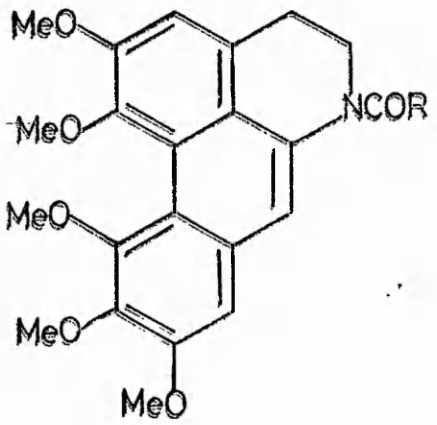
195



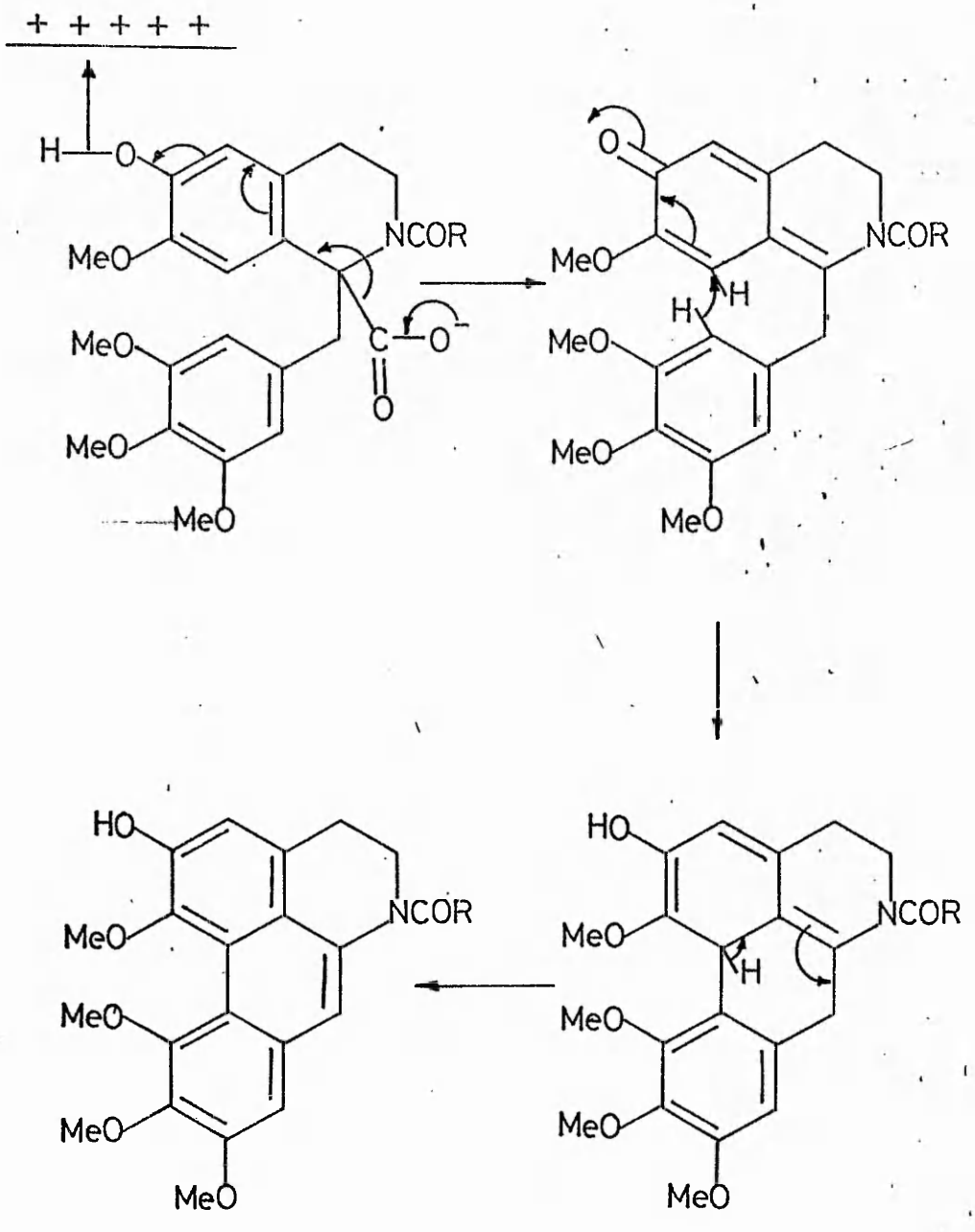
196



198



200



199

SCHEME XVIII

oxidations of 198.

3.1 Synthesis of Aporphine Alkaloids

Aporphine alkaloids constitute one of the largest sub-groups of naturally occurring alkaloids[246,247] and consequently a variety of synthetic methods for preparing such compounds has been developed. The field of aporphine alkaloid synthesis has been extensively reviewed[122,248-250] and it is therefore intended to restrict this discussion to a brief outline of the main synthetic methods, and to cover in greater detail the more promising recent developments which have been published.

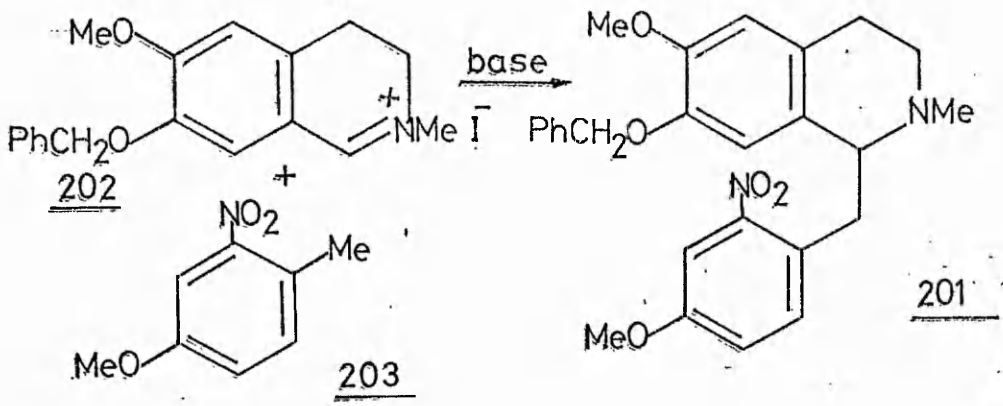
The utilisation of four major synthetic routes, viz, the Pschorr reaction, oxidative coupling of bisphenolic benzylisoquinolines, photochemical- and benzyne-mediated cyclisations, has allowed a wide variety of natural aporphine alkaloids and analogues to be prepared. Although these reactions are still used they generally suffer from poor yields.

The Pschorr ring closure reaction, the original route to aporphines, depends upon the formation of a carbonium ion by the copper-catalysed decomposition of 2'-diazotised benzylisoquinolines. The required aminated isoquinolines were at first prepared by direct nitration and reduction of preformed isoquinolines, but more elegant methods have been developed. Bischler-Napieralski cyclisation of appropriately substituted nitro-amides (produced from β-phenylethylamines and 2-nitrophenylacetic acid derivatives) gives the corresponding 2'-nitro-benzylisoquinolines, which can be converted into aporphines by suitable reduction and Pschorr cyclisation.[251] The Bischler-Napieralski route is generally unsatisfactory if the amide lacks an activating substituent on the aromatic ring of the N-phenylethyl moiety.[228] An alternative

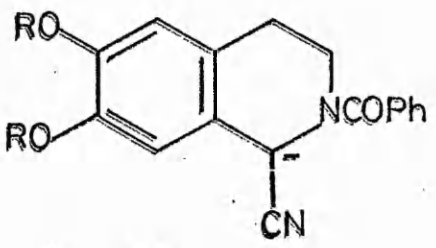
approach utilises the base-catalysed condensation between di- or tetrahydroisoquinolinium salts and 2-nitrotoluenes, e.g.[252] 201 from 202 and 203.

Another variation in preparing the 2-nitrobenzyl precursors, involves the intermediacy of Reissert compounds.[253] Addition of a suitably substituted 2-nitrobenzyl halide to Reissert anions of type 204, formed in the presence of strong base, produces nitro Reissert compounds 205, which on base hydrolysis results in the corresponding 1-benzyl-isoquinolines. Such a procedure has been used by Neumeyer[254,255] in particular, as well as other workers,[256,257] to prepare unusually substituted aporphine alkaloids; (the role of Reissert compounds in isoquinoline alkaloid synthesis has been reviewed[258]). In general, yields of aporphine alkaloids via Pschorr ring closure are low,[259] as other products are possible depending upon the point of ring closure (e.g. 206 ⟶ 76, 207 or 208), and decrease with increasing oxygenation of the isoquinoline. Higher yields are obtained when a bulky substituent is present on the nitrogen atom,[260,261] or when a hydroxy function is present at the C-7 position of the 1-benzyl precursor.[262]

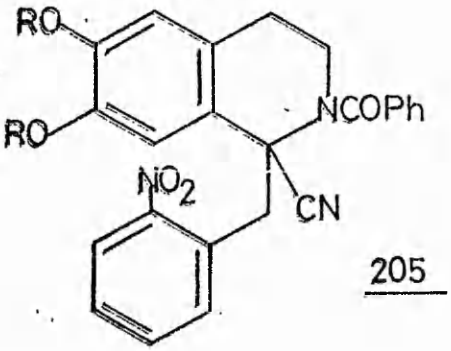
Heating of the diazonium salts formed from 2-aminobenzylisoquinolines, in the absence of a metal catalyst has also been reported[263] to give aporphine compounds. The quinonoidal aporphine 209 has been obtained in 55% yield by Pschorr ring closure[264] and probably results from the aerial oxidation of the corresponding phenolic analogue. A modified Pschorr cyclisation with the amino function situated on ring A rather than on ring D has also been used for aporphine alkaloid synthesis. Irradiation of a diazonium salt in dilute acid, i.e. a photo-Pschorr reaction, has been used to prepare such alkaloids, although only a 2%



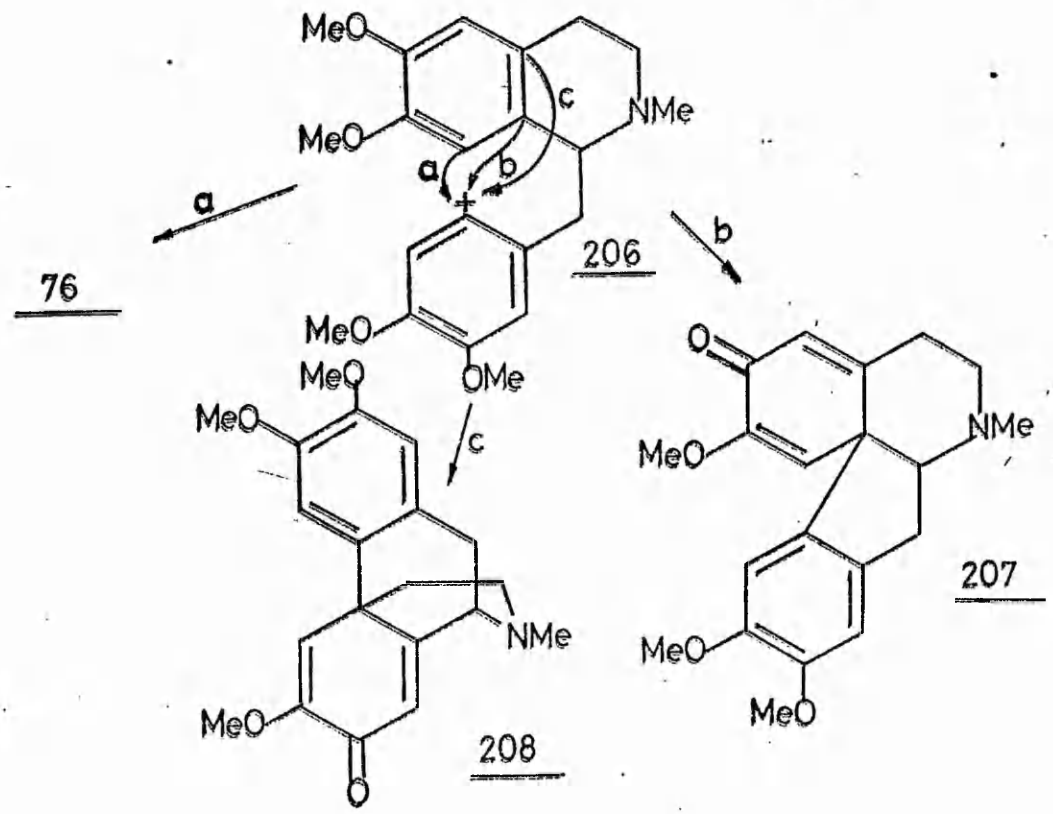
202 203 201



204



205



208 207

yield of aporphine was obtained.[265]

The production of benzyne intermediates from 2'- or 3'-halogenobenzylisoquinolines by the action of a strong base (e.g. alkali metal amides in ammonia, or potassium tert-butoxide) also leads to aporphine alkaloid formation. Ring closure proceeds efficiently only if the aromatic ring is rendered strongly nucleophilic by anionic activation towards the formed benzyne. Kessar and his co-workers[266] have established that a benzyne mechanism operates in potassamide-ammonia, rather than alternative mechanisms involving either intramolecular halogen displacement by the activated C-8 atom, or radical nucleophilic substitution. This view is supported by the finding that both phenolic 2'- and 3'-halogenated substrates furnish aporphines in similar yields.[267] In the absence of an activating phenolic moiety starting material and the dehalogenated product were recovered.[266] Gibson[268] has shown, using 2'-bromolaudanosine in metal amide-ammonia, that 2'-aminolaudanosine is formed in small amounts along with the major indole-type products. Such an aminated product is presumably formed by nucleophilic attack of the amide anion on the initially formed benzyne intermediate. Pschorr ring closure of this amino product led to the required aporphine alkaloid. Formation of other structurally different products e.g. morphinandienones[269] and dibenzopyrrocoline-type[269,270] compounds, *via* a benzyne route has been demonstrated, and consequently yields of aporphine products by this method are often low. Syntheses of 6a,7-dehydroaporphines through the intermediacy of benzyne intermediates, using sodium methylsulphinylmethanide as the base, have also been reported.[271,272]

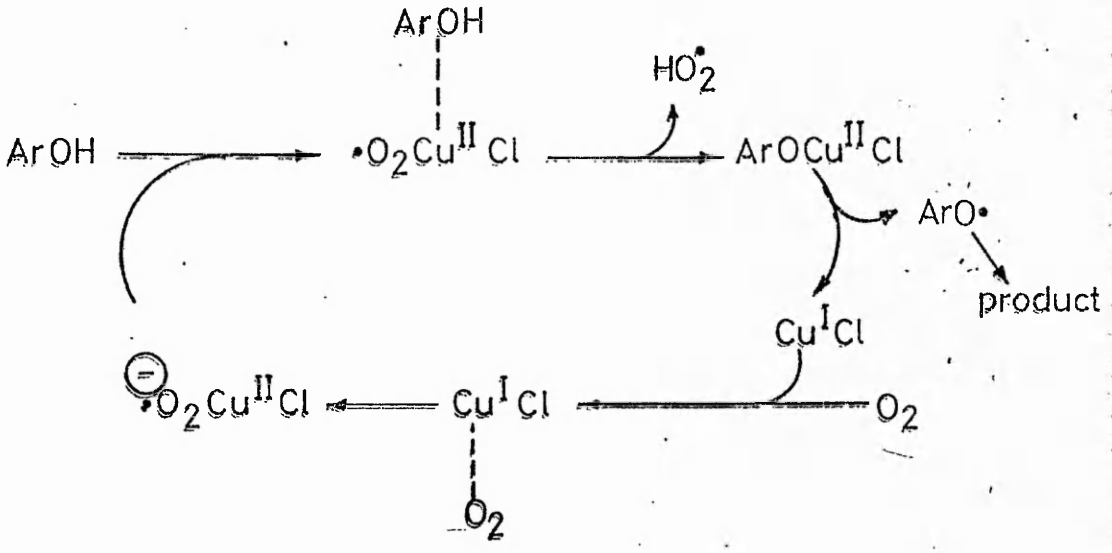
Phenol oxidative coupling by chemical oxidants, e.g. $K_3Fe(CN)_6$, $FeCl_3$ and MnO_2 to give directly aporphine alkaloids has long been known, but

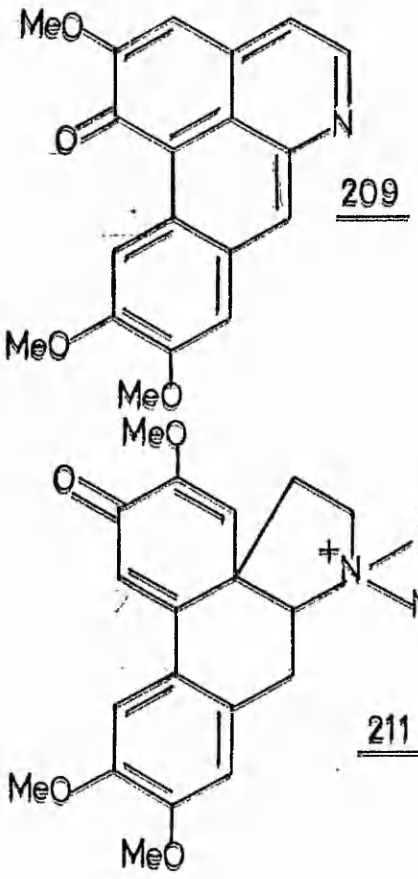
unfortunately usually give poor yields. Other oxidants such as vanadium oxychloride[273] and thallic trifluoroacetate[274] have recently come into prominence in this field, and yields considerably better than most of the former set of reagents have been recorded. Recent studies by Kametani[275,276] have demonstrated that a mixture of cuprous chloride and oxygen in pyridine can oxidise (+) reticuline both by ortho-ortho and ortho-para modes to give (+) corytuberine and (+) isoboldine in 28 and 8% yield respectively. This process has been likened to the oxidation of phenolic isoquinolines by copper-containing enzymes, e.g. laccase. Scheme XIX represents the possible interaction between the copper atom, the phenolic substrate and an oxygen molecule.

The application of phenol oxidative coupling to the synthesis of aporphine alkaloids (i.e. by direct coupling, by proaporphine and neo-proaporphine formation), has previously been dealt with (see Section 1.2) It is only recently that acid-catalysed rearrangement of neoproaporphine systems to aporphine structures has been achieved; Kupchan[277] has succeeded in rearranging the borane complexes of the dienones 210, 211 and 212 to aporphine alkaloids in good yields. Acid catalysed rearrangement of the procularine compounds 91 gave[278] an aporphine product rather than the expected cularine compound. Transformation of the dienol 213 to the aporphine alkaloid 214 has been achieved[279] using methyl fluorosulphonate, although the yield was less than 1%. Similarly, acid-induced rearrangement of a mixture of the epimeric dienols 215 gave 1,10-dimethoxy-9-hydroxyaporphine in 85% yield.[280]

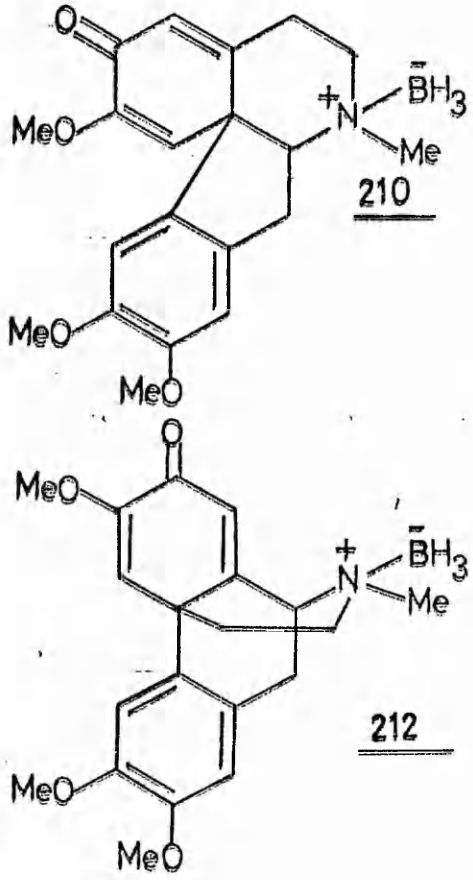
Photochemical cyclisations have over the past decade, played an important role in aporphine alkaloid synthesis[281] and of particular importance is the modified version of the stilbene-phenanthrene transformation, developed by Cava,[282] involving the non-oxidative

SCHEME XIX





209



210

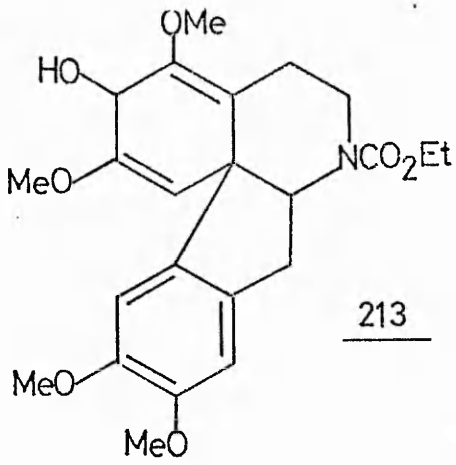
211

212

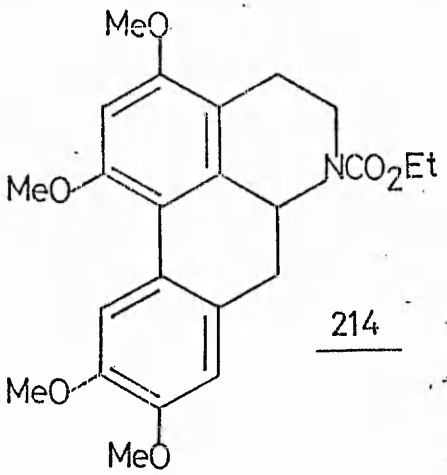
irradiation of halogenostilbenes of type 216, in the presence of potassium tert-butoxide. Yields of 6a, 7-dehydroaporphines by such a process are high[283,284] (over 70% in certain cases[282]). The subject of enamide photolysis has been reviewed[285]. Synthesis of aporphine alkaloids by photolysis of either halogenated or non-halogenated tetrahydroisoquinoline precursors has also been reported.[286-8]

Non-oxidative photolysis of the N-acetyl-2'-chlorobenzylidene isoquinoline 217 was found[289] to be a more rewarding route to the aporphine alkaloid elmerrillicine via a 6a,7-dehydroaporphine than either Pschorr cyclisation of the appropriate 2'-amino compound, or photolysis of the N-acetyl-2'-iodo-1,2,3,4-tetrahydroisoquinoline 218. Japanese workers[290] have successfully prepared the aporphine domesticine in 31% yield by irradiation of a phenolic bromoisoquinoline in the presence of sodamide and DMF; although the authors implicate phenyl radical attack on the activated ring, a benzyne-mediated mechanism could also operate. Shamma has reported[291] the preparation of de-N-methylthalphenine 219 which may result from the conversion of the phenolic aporphine 220, through the quinone methide 221, to the aporphine ether 219 via rapid bond isomerisation.

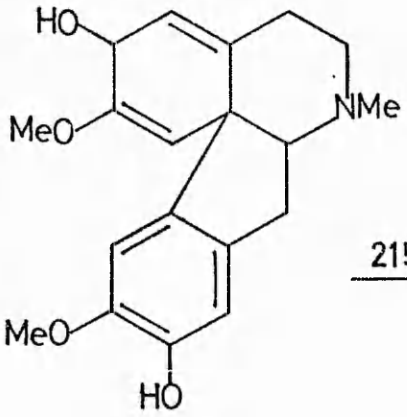
Recently the use of certain one- and two-electron chemical oxidants has enabled non-phenolic and monophenolic isoquinolines to be converted efficiently to aporphine alkaloids. Lead tetraacetate oxidations have been particularly effective in aporphine synthesis[292] and have been reviewed.[293] In order to activate the D ring of benzylisoquinolines towards cyclisation it was found necessary to have an oxygen function para to the reacting site. Oxidation of 7-hydroxybenzylisoquinolines by lead tetraacetate initially produces p-quinol acetates having the general structure 222 which upon acidification rearrange to the corres-



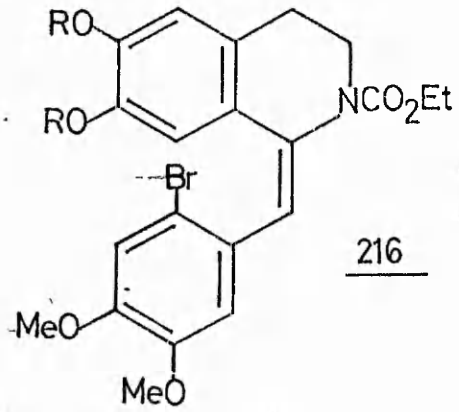
213



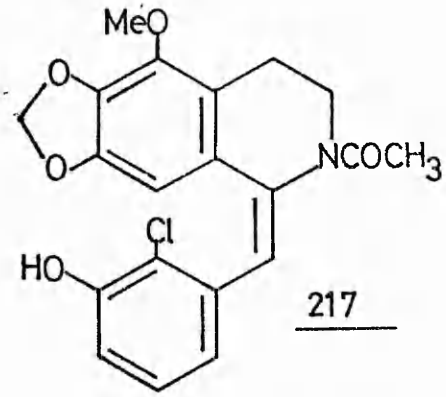
214



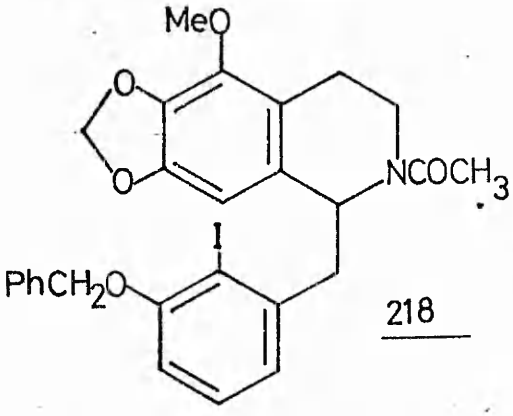
215



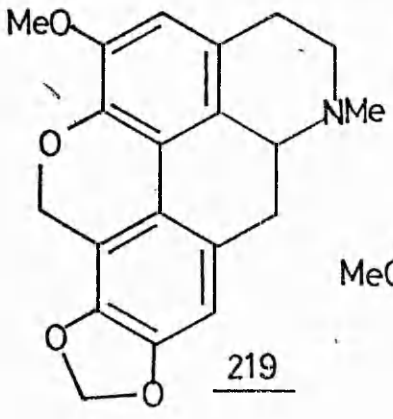
216



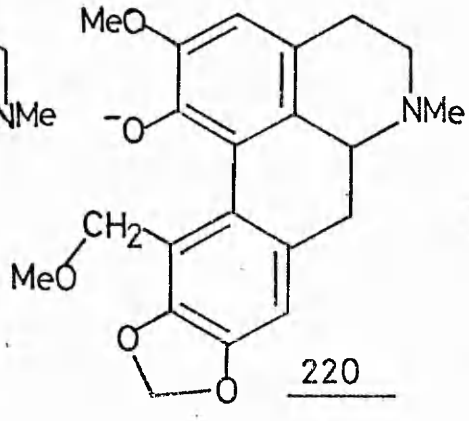
217



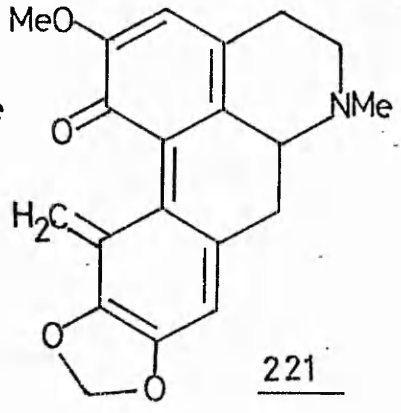
218



219



220

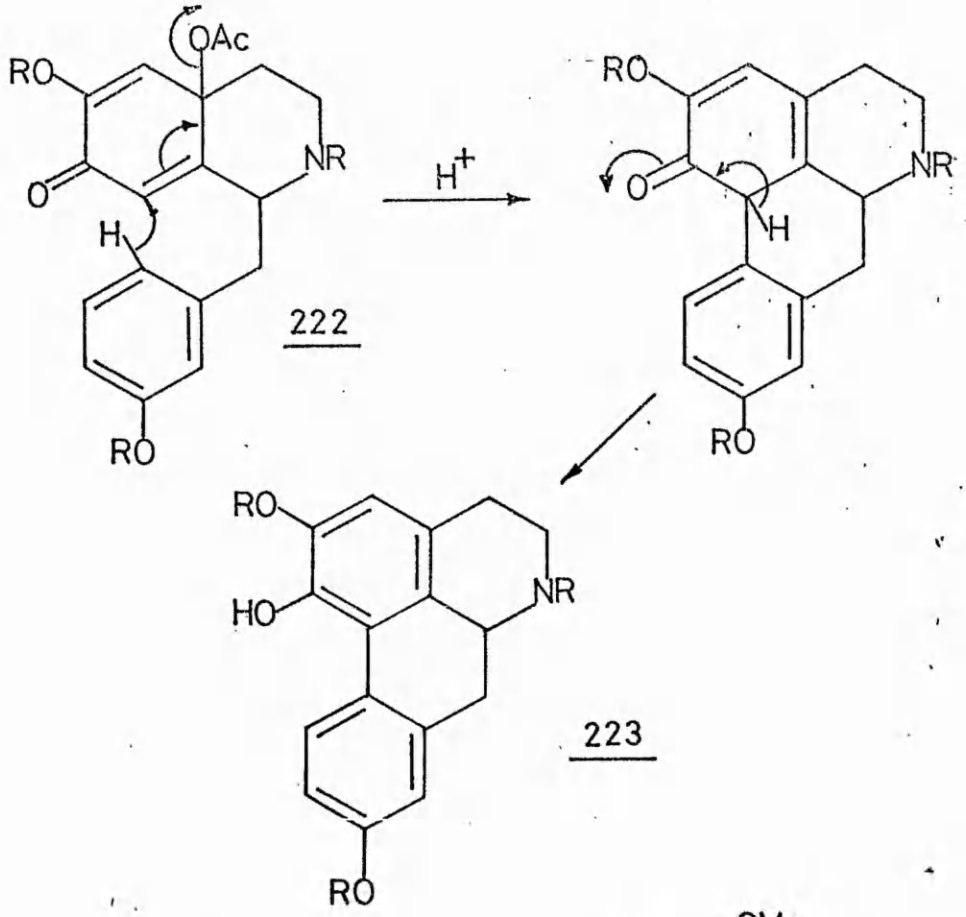


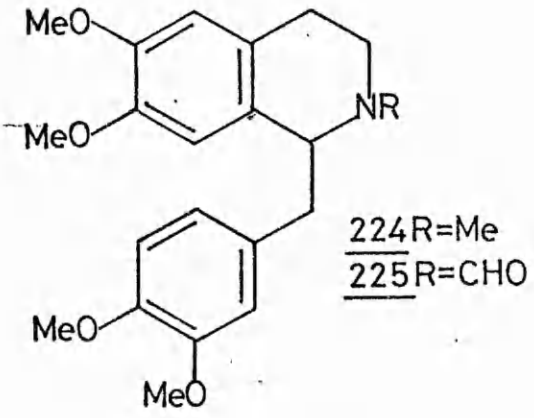
221

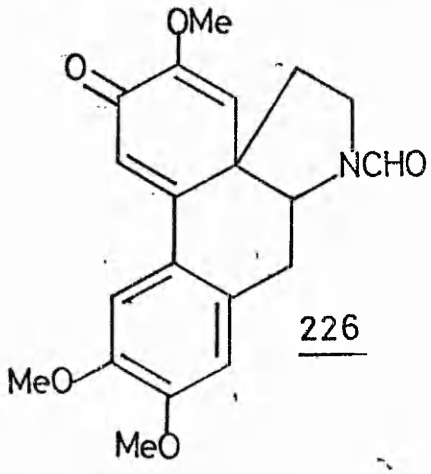
ponding aporphine alkaloids by the mechanism shown, i.e. 222 → 223. Yields by this route are in general good, compared with some other chemical methods. Preparation of aporphines by the action of vanadium oxytrifluoride on monophenolic isoquinolines in trifluoroacetic acid have also been successful, giving high yields of aporphine alkaloids.[294] Lower yields were obtained when the nitrogen lone pair electrons were unblocked. Conversely, it was found that vanadium oxytrifluoride oxidation of non-phenolic isoquinolines gave better yields when the lone pair electrons were available,[295] e.g. 224 gave 43% of the appropriate aporphine, whereas only 6% of aporphine product was obtained from the N-formyl analogue 225, the major product in this case being the spirodienone 226. An investigation[264] into the oxidation of 227 by various chemical reagents has demonstrated the efficiency with which vanadium oxytrifluoride and molybdenum oxytetrachloride are able to bring about intramolecular cyclisations, furnishing the quinonoidal oxoaporphine 209 in 59 and 62% yield respectively.

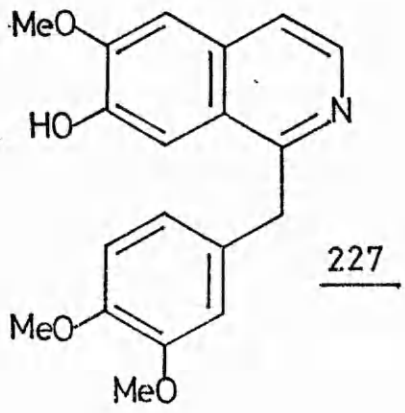
Thallium tris(trifluoroacetate), has been used by Taylor and his collaborators[296] to prepare thalicmine 228 in 46% yield by a one-step non-phenolic coupling reaction of an appropriate precursor. The same compound was prepared in only 11% yield by a Pschorr ring closure.[297] An interesting observation from this same work was the formation of an acetoxylated aporphine 229 by treatment of the same precursor with thallium III acetate. This was the first reported formal phenolic functionalisation of the D ring in an aporphine alkaloid.

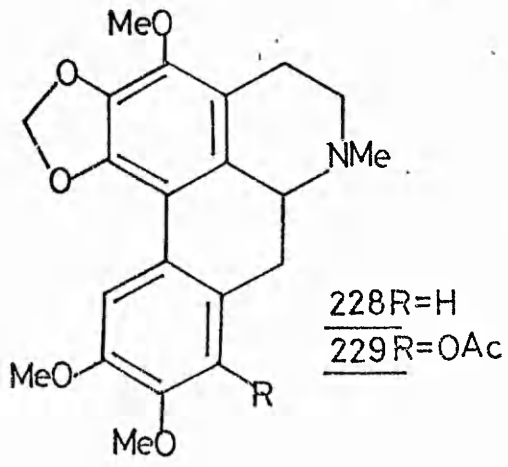
The use of enzymes for the synthesis of aporphines and other isoquinoline alkaloids has been discussed (see Section 1.4.2), and has recently been reviewed.[298] Other miscellaneous routes to aporphine alkaloids have


OAc
RO
NR
O
H
H+
222
223
HO


MeO
NR
224 R=Me
225 R=CHO

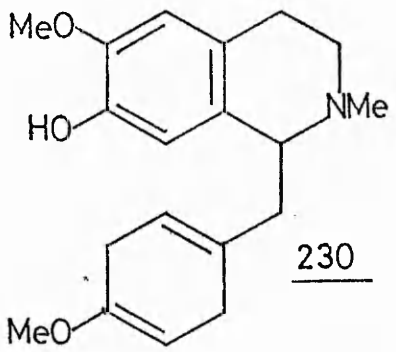

OMe
O
NCHO
MeO
226


MeO
HO
N
227

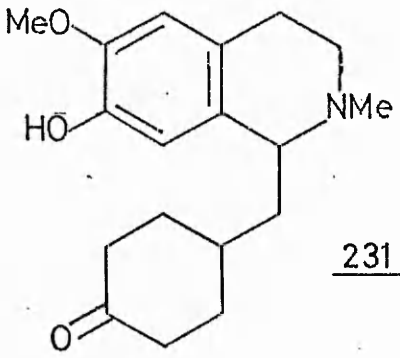

MeO
O
NMe
R
228 R=H
229 R=OAc

also been reported. The cyclohexadiene derivative 230 has been converted into the aporphine 231 by the action of phosphoric acid;[299] similarly the Grewe cyclisation of the oxalate of 1-(4-hydroxybenzyl)-2-methyl-1,2,3,4,5,8-hexahydroisoquinoline 232, in 85% phosphoric acid gave[300] both 233 and 234 in approximately equal proportions, possibly *via* disproportionation of the initially formed octahydro-aporphine 235. Electrochemical oxidation of laudanosine was found to produce the morphinandienone 236 quantitatively and this in turn was converted[301] in 90% yield to the hydroxy aporphine 237. Similarly the isomeric dienone 238 gave the expected aporphine 75 in 31% yield. Anodic oxidation of laudanosine in trifluoroacetic acid afforded glaucine 76 in 17% yield.[301] A novel approach[302] to aporphine compounds involves the cathodic cyclisation of an iodobenzylisoquinolinium salt (prepared *via* Reissert alkylation) in acetonitrile, to give an 86% yield of the 4,5,6a,7-tetra-dehydroaporphine 239. The dimethoxy analogue 240 was prepared in a similar manner in 74% yield.

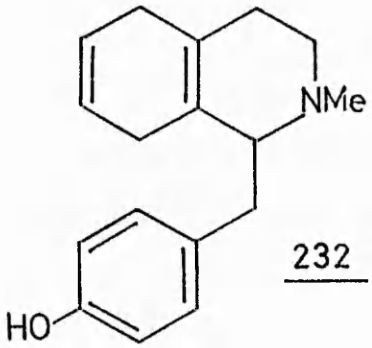
The oxidation of non-phenolic aporphine alkaloids to their corresponding 6a,7-dehydroaporphines has been achieved by a variety of oxidising agents, including potassium permanganate,[303] DDQ,[304] mercuric chloride[305] and iodine,[306] the last of which has been shown to give better yields of the dehydrogenated product. More recently, very high-yield conversions of aporphines to dehydroaporphines have been achieved using 10% palladised charcoal in refluxing acetonitrile.[307] It was also observed that noraporphines and phenolic aporphines gave oxoaporphines by aerial oxidation. Low yields of dehydroglaucine 241 have also been obtained from glaucine by lead tetraacetate oxidation.[308] Similarly the formation of the oxoaporphine 242 by u.v. irradiation was assumed[309]



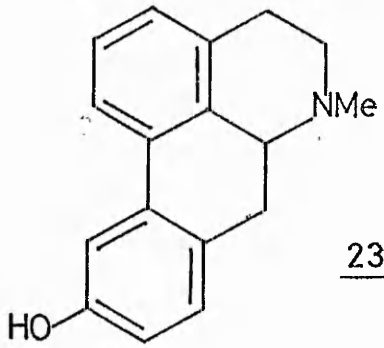
230



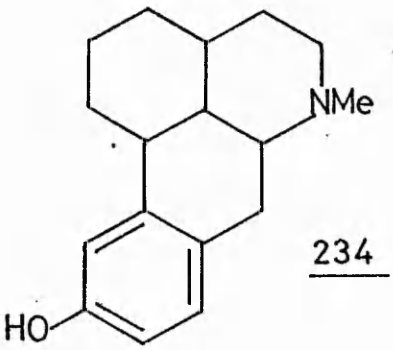
231



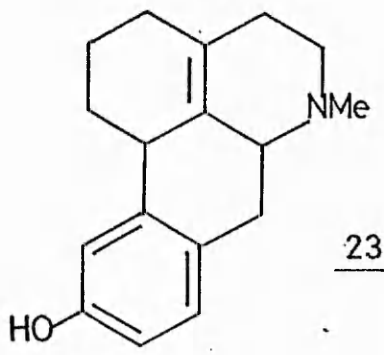
232



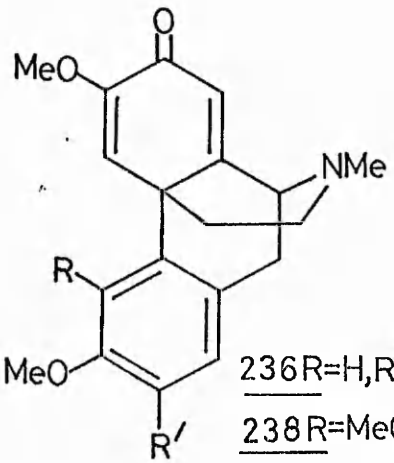
233



234

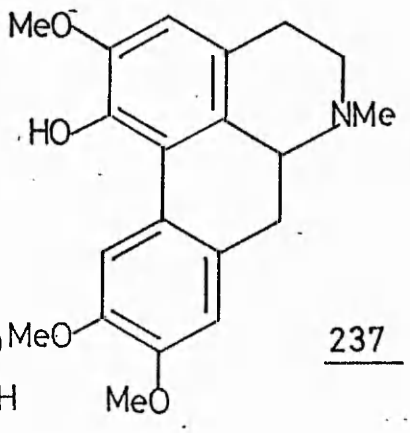


235



236 R=H, R'=MeO

238 R=MeO, R'=H



237

to involve the initial formation of a 6a,7-dehydroaporphine 243 followed by loss of the N-methyl group (possibly as carbon dioxide), and double bond migration to the 6a- -dehydroaporphine 244 with subsequent oxidation.

3.2 Synthesis of Substrates

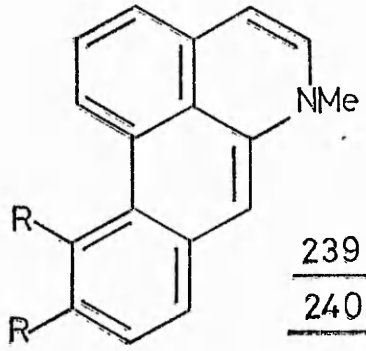
3.2.1 N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 245

Attempts to prepare N-acylated derivatives of the ∝-aminoacid 166 using acetyl chloride,[1] ethyl chloroformate or freshly prepared acetic-formic anhydride,[310] proved unsatisfactory, giving low yields of unidentifiable products. Results using trifluoroacetic anhydride were considerably better, high yields of the novel N-trifluoroacetylated acid 245 being obtained on stirring acid 166 with a small excess of the anhydride at room temperature in the presence of triethylamine. In view of this finding the trifluoroacetyl moiety was chosen as the protecting group for the nitrogen atom throughout this study.

The attempted preparation of 245 using trifluoroacetic anhydride in refluxing pyridine gave high yields of a neutral compound. This anomalous reaction will be discussed in Chapter 5 (Section 5.3)

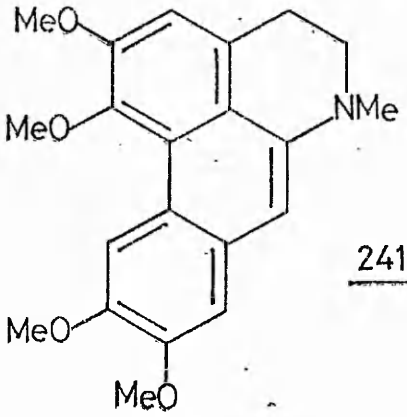
3.2.2 6a,7-Dehydroaporphine and Aporphine Compounds Corresponding to 245

In consideration of the high yields of 6a,7-dehydroaporphines obtained *via* the irradiation of bromostilbenes in the presence of potassium tert-butoxide and tert-butanol,[282,283] the preparation of the required pentamethoxy-6a,7-dehydroaporphine 246 was attempted by such a route.

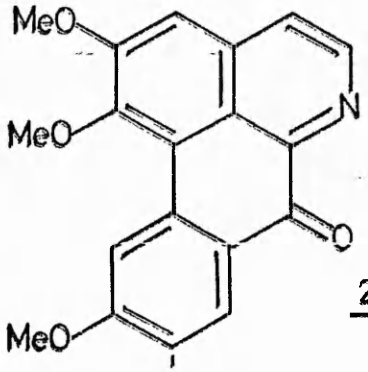


239 R=H

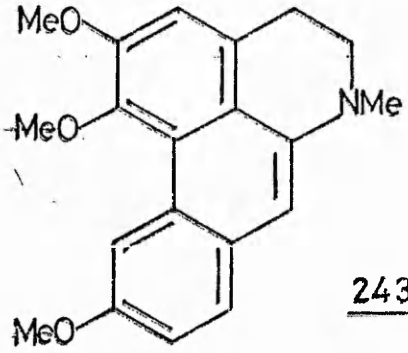
240 R=MeO



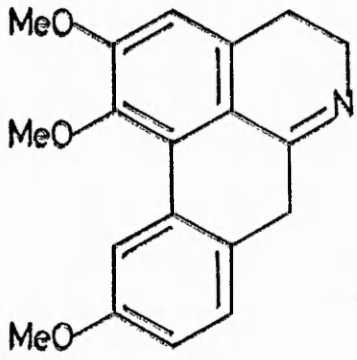
241



242



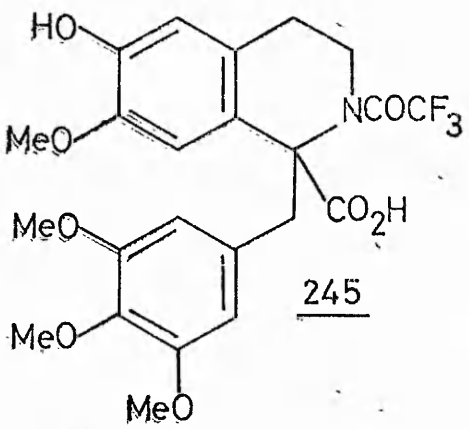
243



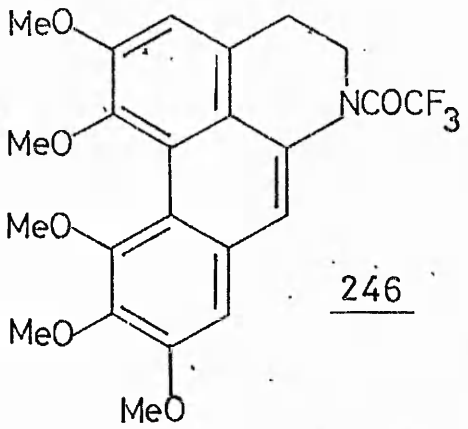
244

Bromination of 3,4,5-trimethoxyphenylacetic acid according to the method of Kametani,[311] gave 2-bromo-3,4,5-trimethoxyphenylacetic acid 247 in 91% yield. Earlier attempts to make the same product using undistilled chloroform as the solvent resulted in the formation of the ethyl ester 248, presumably by hydrogen bromide catalysed esterification between the brominated acid and the ethanol present in chloroform. Preparation of the corresponding amide 249 from 247 and homoveratrylamine using N,N'-dicyclohexylcarbodiimide to promote coupling proved unsuccesful, giving instead a mixture of the mono and dibrominated adducts 250 and 251; the latter was the minor component, probably arising from 2,6-dibromo-3,4,5-trimethoxyphenylacetic acid present as a trace impurity in 247. These two ureas showed distinctive i.r. spectra, each having two C=O and one N-H absorptions; their nmr spectra exhibited a high field multipletdue to the cyclohexyl groups. Amide 249 was successfully synthesised by heating at 180° the salt obtained from homoveratrylamine and acid 247.

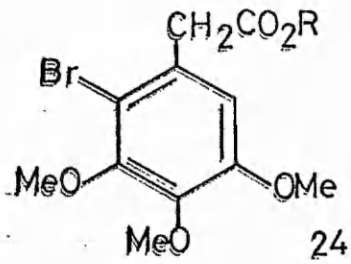
Ring closure of the amide using phosphorus oxychloride according to the method described by Cava,[283] gave a low yield of 1-(2'-bromo-3',4',5'-trimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline 252, but it was obtained in 61% when cyclisation with phosphorus pentachloride[312] was used. N-Trifluoroacetylation of 252 with trifluoroacetic anhydride in chloroform produced the required enamide 253. Although compound 253 is illustrated in the cis-stilbene form, an equal mixture of the cis and trans isomers was produced, exhibiting two distinct singlet absorptions in the ^{19}F nmr, arising from the deshielded cis and shielded trans structures; (a similar deduction was made by PCMU, based upon the ^{13}C nmr spectrum for this compound). Trifluoroacetylation carried out in pyridine gave a mixture of both 253 and a pale yellow compound which



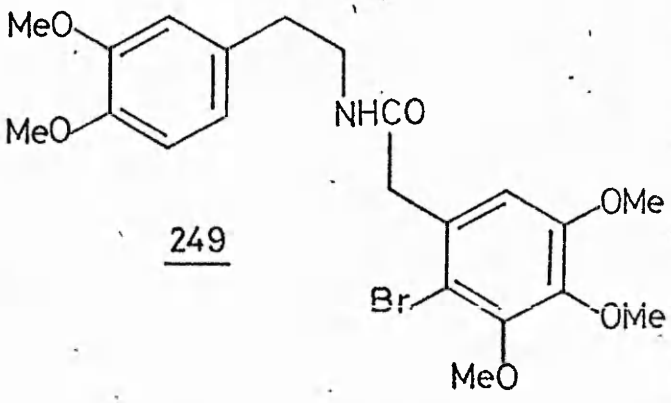
245



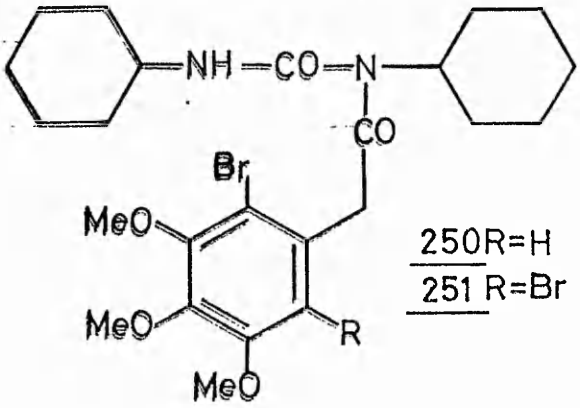
246



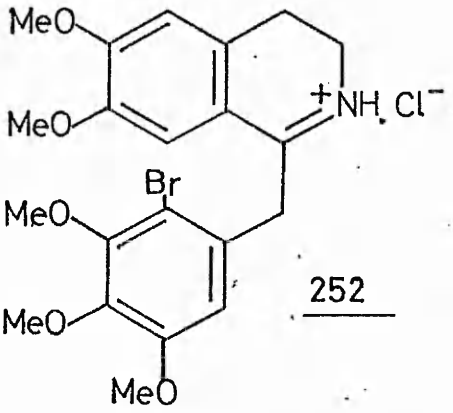
247 R=H
248 R=Et



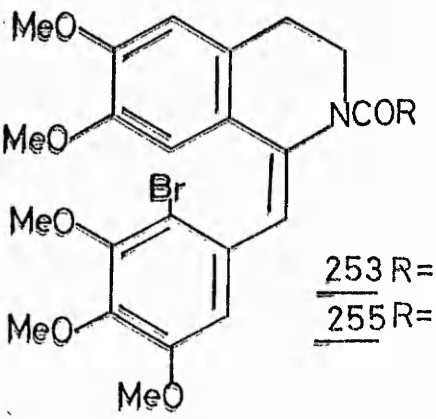
249



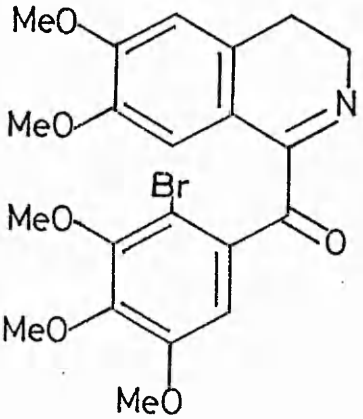
250 R=H
251 R=Br



252



253 R=CF3
255 R=OEt



254

gave no ^{19}F nmr signal and had an infrared spectrum showing a carbonyl band at 1660 cm^{-1}, rather than the band at approx. 1700 cm^{-1} characteristic of a trifluoroacetamide. Based upon this and mass spectral and nmr data the compound was identified as 1-(2'-bromo-3',4',5'-trimethoxybenzoyl)-6,7-dimethoxy-3,4-dihydroisoquinoline 254.

When the N-trifluoroacetyled product 253 was dissolved in benzene containing potassium tert-butoxide and tert-butanol a yellow solution was immediately obtained. T.l.c. showed the absence of 253 and the presence of a spot which was coincident with the keto-imine 254. Isolation of the product confirmed that 254 was indeed formed by the immediate deacylation and oxidation of 253. Irradiation of 254 was attempted in the hope that cyclisation of an ∝-oxoisoquinoline would give an oxoaporphine, similar to the results reported by Kametani[313]. However no such product was formed, with only starting material being recovered. Irradiation of 253 in non-basic media using either methanol or benzene as solvent and calcium carbonate as the required hydrogen halide scavenger produced intractable mixtures.

In view of the ease with which the trifluoroacetyl function is removed in basic media, it was next decided to employ the N-ethoxycarbonyl blocking group, as used by Cava[283] and his co-workers. The corresponding N-ethoxycarbonyl aminostilbene 255 was prepared in 35% yield from 252 and ethyl chloroformate; the nmr spectrum of the product indicated that a preponderance of the trans isomer was present. Photolysis of 255 for 14 hr. was carried out according to the literature method[283] and resulted in only unreacted starting material being recovered. This apparent non-reaction was probably a result of the lower output (90 watt) from the photochemical reactor used, compared with that employed by Cava et al

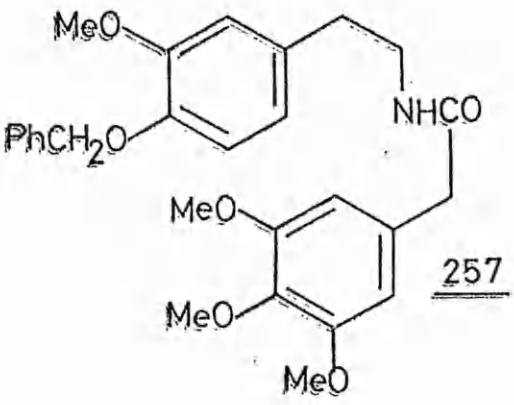
(450 watt). However when this same solution was irradiated for a further 10 days it gave in addition to unreacted starting material a small amount of a yellow oil which could not be made to crystallise; this could have been the required dehydroaporphine in view of an absorption band at 260-270 nm in its u.v. spectrum (characteristic of dehydroaporphine.[122]) This product was not examined further because of the success of other synthetic methods (see later).

Electrochemical reduction of a compound structurally similar to 253 has been reported to give a 6a,7-dehydroaporphine.[302] An attempt to reproduce such a reaction at a carbon felt cathode, with 253 as substrate was made. Unfortunately difficulties were experienced in maintaining a sufficiently high working current, presumably as a result of electrode coating. This approach was not continued.

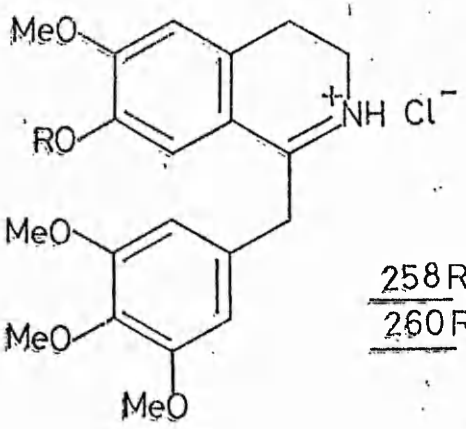
The use of lead tetraacetate[292] and vanadium oxytrifluoride[294] have recently been reported to effect the ring closure of 7-hydroxy-1-benzylisoquinolines to the appropriate 1-hydroxyaporphines. It was therefore considered that cyclisation of the N-trifluoroacetyl-1-benzylideneisoquinoline 256 with either lead tetraacetate or vanadium oxytrifluoride and subsequent methylation represented a reasonable approach to the 6a,7-dehydroaporphine 246. The known amide[292] 257 and 3,4-dihydroisoquinoline[292] 258 were prepared by heating the appropriate phenylethylamine salt and cyclisation with phosphorus oxychloride respectively. Treatment of 258 with trifluoroacetic anhydride in the usual manner gave the novel N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzylidene)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 259. Catalytic hydrogenolysis of this benzyl ether then furnished the required phenolic precursor 256. An alternative

route to the same compound was to debenzylate 258 and to react the resultant 1-(3',4',5'-trimethoxybenzyl)-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline 260 with trifluoroacetic anhydride. The major product given by this method was almost certainly the O,N-bistrifluoroacetyl compound 261, showing two distinctive carbonyl absorptions at 1800 and 1685 cm^{-1}, indicative of $-OCOCF_3$ and $-NCOCF_3$ respectively. Attempts to obtain 261 in a pure state by both column and thick layer chromatography resulted in cleavage of the depside bond, furnishing the desired phenolic compound 256. Hydrolysis of 261 in 10% sodium carbonate solution gave two products, the phenolic benzylidene 256 and 1-(3',4',5'trimethoxybenzoyl)-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline 262 in equal amounts. The attempted cyclisation of 256 using lead tetraacetate and vanadium oxytrifluoride according to the reported methods, gave the same product in both cases, identified as the keto-imine 262. It would therefore seem that both of these reagents interact in some way with the unsaturated function of 256, inducing the molecule to deacylate and to be oxidised to the keto-imine structure, although the sequence of these two reactions is not known.

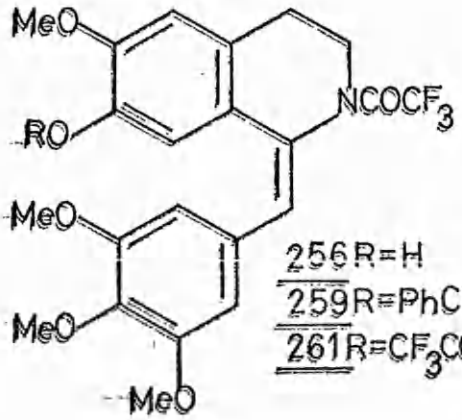
The cyclisation of N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline 263 was next attempted. Reduction of the dihydro compound 258 to the tetrahydroisoquinoline 264 was found to be unsatisfactory using sodium borohydride and so was achieved by a refluxing zinc-glacial acetic acid mixture. Reaction of 264 with trifluoroacetic anhydride gave N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 265 in 47% yield which upon catalytic hydrogenolysis furnished the required phenolic compound 263.



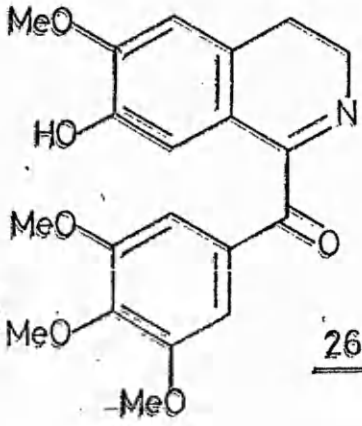
257



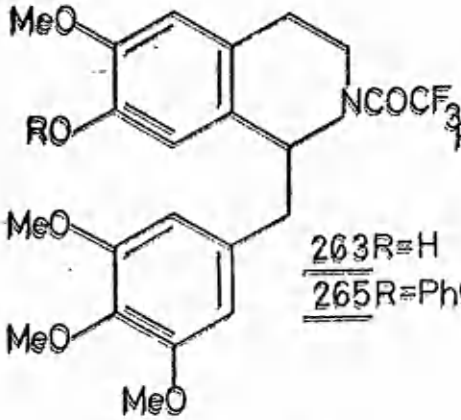
258 R=PhCH2
260 R=H



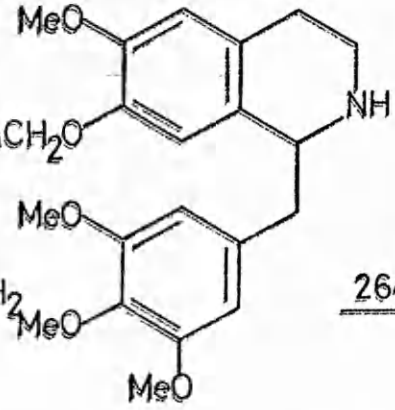
256 R=H
259 R=PhCH2
261 R=CF3CO



262

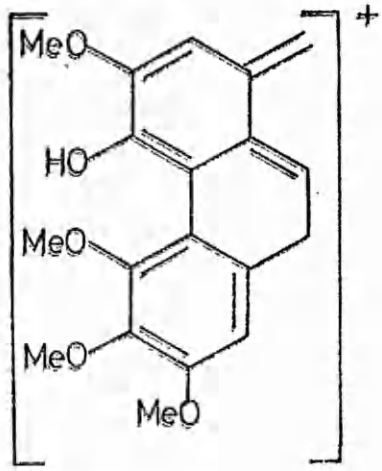


263 R=H
265 R=PhCH2



264

266 R=H
269 R=Me

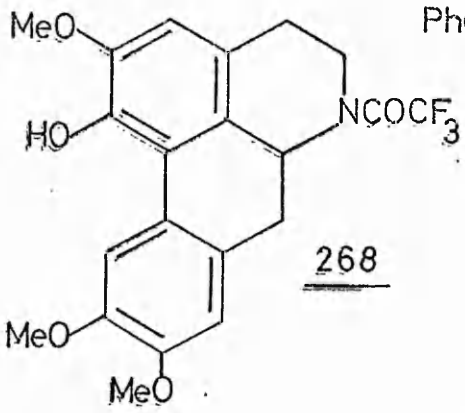


267

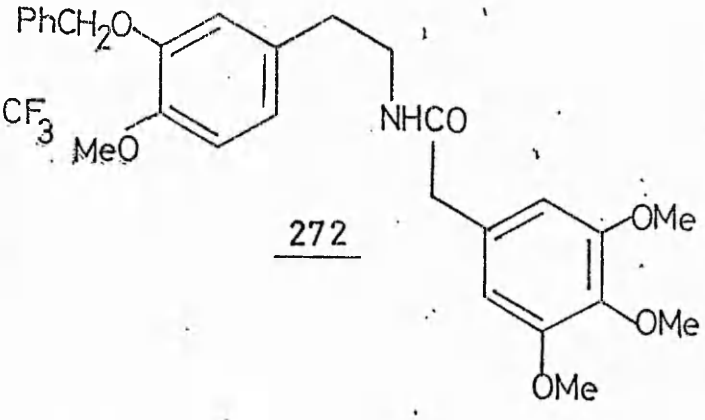
The novel aporphine 266 was prepared in 35% and 10% yields using lead tetraacetate and vanadium oxytrifluoride respectively. The base peak in the high resolution mass spectrum of 266 had an m/e value of 327; indicative[122,314] of the fragment ion 267, a retro Diels-Alder type species, characteristic of aporphine structures. The lower yield obtained from vanadium oxytrifluoride was disappointing, since Kupchan[294] had reported a 70% yield of N-trifluoroacetylwilsonirine 268 from the appropriate N-trifluoroacetyl precursor by this method and a yield at least comparable to this was anticipated since 263 differed from Kupchan's starting material only by an additional methoxy group. However the difficulties in handling this extremely hygroscopic reagent could have caused the lower yield. The yield of aporphine alkaloid from the lead tetraacetate cyclisation was in agreement with those recorded by Umezawa.[292]

Methylation of 266 could not be carried out using diazomethane, iodomethane-potassium carbonate or dimethylsulphate-potassium carbonate, presumably because of steric hinderance of the 1-hydroxy group by the surrounding methoxy groups, but was effected using a mixture of sodium hydride and iodomethane in DMF, affording N-trifluoroacetyl-1,2,9,10,11-pentamethoxy-noraporphine 269 in 65% yield. Several attempts to oxidise this pentamethoxy aporphine using iodine in dioxan[306] failed to produce the expected 6a,7-dehydroaporphine 246.

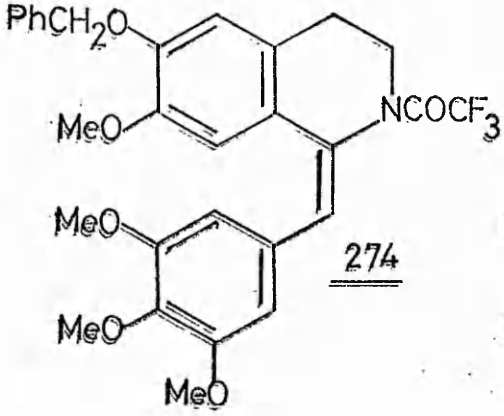
It was also considered possible that the 2-hydroxy-6a,7-dehydroaporphine 270 could be prepared from N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzylidene)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 271 by treatment with DDQ, with the reaction proceeding by the mechanism shown in Scheme XX. This route appeared more attractive than the use of the 7-hydroxy



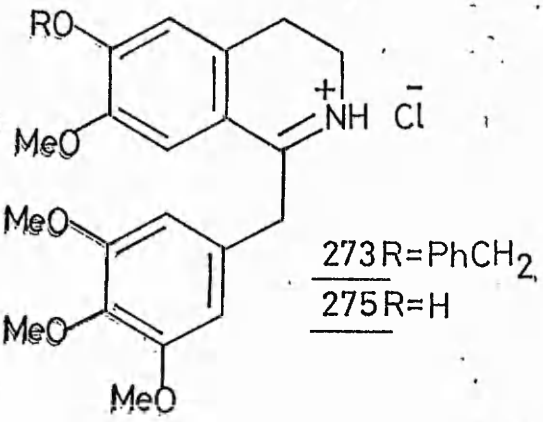
268



272

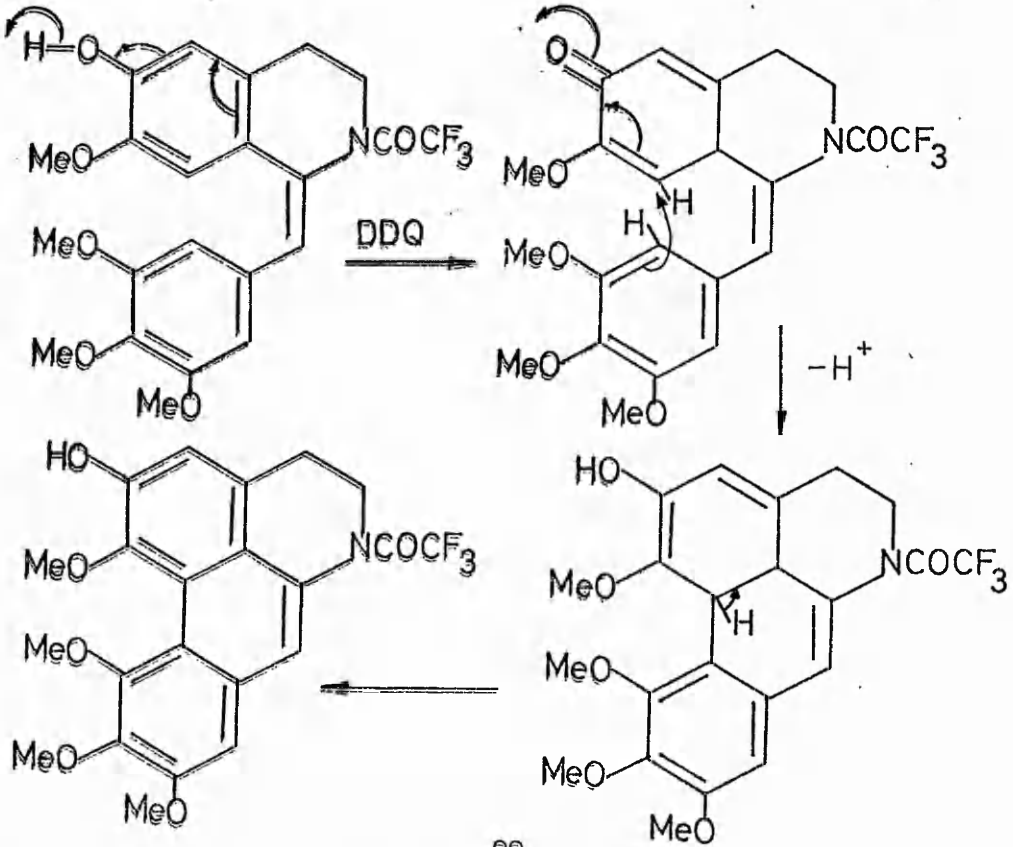


274



273 R=PhCH2
275 R=H

SCHEME XX



271

270

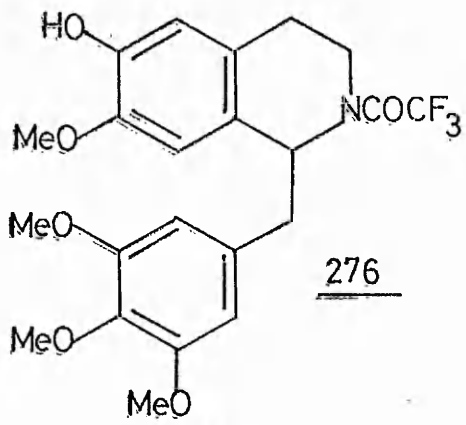
precursors because it would give directly the correctly substituted 6a,7-dehydroaporphine postulated in the oxidative decarboxylation of amido-acid 245. Compound 271 was prepared by the same route as its 7-hydroxy analogue through the novel amide 272, 3,4-dihydroisoquinoline hydrochloride 273 and N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzylidene)-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 274. It was also found as with the 7-hydroxy series that attempts to prepare 271 from the phenolic dihydroisoquinoline 275 gave a compound which exhibited carbonyl absorptions at 1810 and 1700 cm^{-1} in the i.r. spectrum, inferring an O,N-ditrifluoroacetylated product. Reaction of 271 with an equimolar amount of DDQ in benzene gave an intractable gum as the product and was not pursued further. A similar attempted cyclisation of N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 276 prepared in the previously described manner via catalytic hydrogenolysis of the benzyloxy precursor 277 also failed to give any aporphine product.

3.3. Oxidation Studies on the ∝-Amido-Acid 245

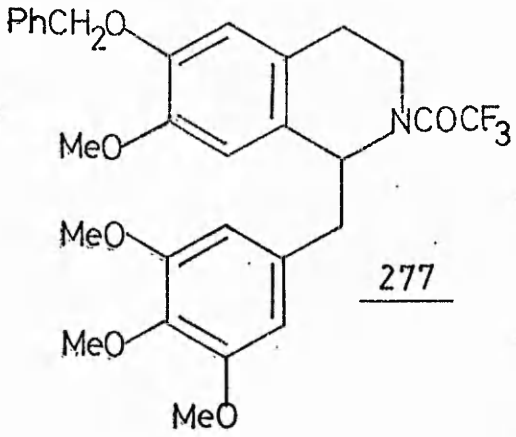
The object of this section of the work was to investigate the possible formation of the 6a,7-dehydroaporphine 270 by the action of a variety of oxidants on the amido-acid 245; an alternative aporphine compound 278 could also possibly arise via the same coupling reaction and a subsequent reductive stage. Unfortunately failure to synthesise either the 2-hydroxy aporphine or the corresponding 6a,7-dehydroaporphine meant that it was necessary in all the following oxidation studies on 245 to methylate and reduce the crude reaction product mixture, thus enabling aporphine-type products to be identified by comparison with the prepared N-trifluoroacetyl-1,2,9,10,11-pentamethoxyaporphine 269.

3.3.1 Anodic Oxidation

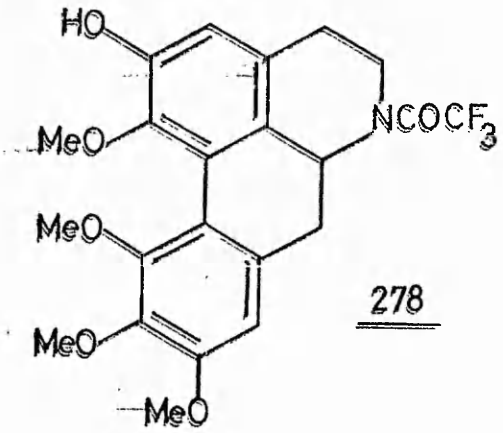
The selection of suitable solvent systems for the electrochemical oxidations of 245 was important in view of the sensitivity of the N-blocking group towards basic media. The two systems chosen were solutions of sodium bicarbonate in either methanol-water[1] or acetonitrile-water, both of which were sufficiently basic to generate the required carboxylate anion, yet would not remove the N-trifluoroacetyl function. Preliminary experiments with these solvents showed that 245 was sufficiently soluble in both solvent systems and could be recovered unchanged by acidification and chloroform extraction, even after standing for 24 hr., thus demonstrating the suitability of these two media for the following electrochemical studies. Anodic oxidations of 245 using the methanol and acetonitrile solvent systems were carried out on carbon felt anodes at applied potentials of 360 and 270 mV respectively, against a standard calomel electrode, until no starting material was detectable by t.l.c. The product mixtures from both reactions were shown to be virtually identical by t.l.c., with the keto-imine 279 identifiable by its characteristic orange colouration on silica-gel as the major component. Confirmation of the identity of this compound was obtained by an independent synthesis in which 279 was obtained in 60% yield by the electrochemical oxidation of the unblocked acid 166, according to the method of Bobbitt.[1] Subsequent methylation by diazomethane and catalytic reduction (PtO_2) of the oxidation medium failed to demonstrate the presence of a component having the same R_f value as the pentamethoxyaporphine 269 using several different chromatographic solvent systems. Although these findings suggest that aporphine-type products are not formed via oxidation of 245, it is possible that either the methylation or reductive stages or



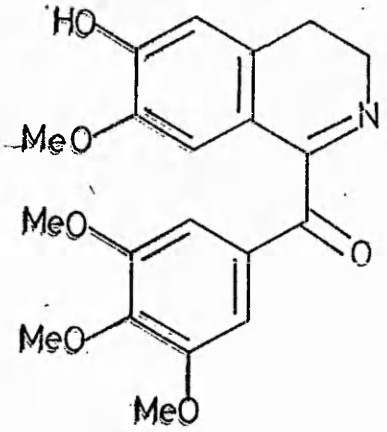
276



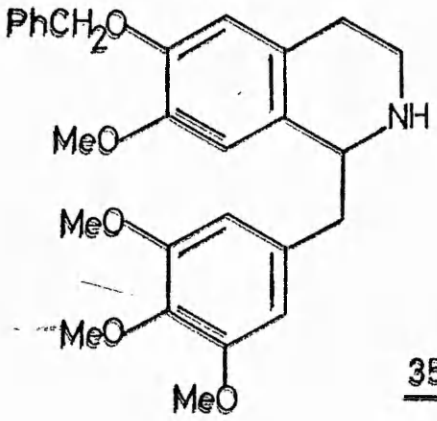
277



278



279



354

possibly both may have been ineffective.

3.3.2 Enzymatic Oxidation

Reaction of 245 with both laccase and horseradish peroxidase-peroxide was carried out in a solution of phosphate buffer (pH6) containing just sufficient ethanol to dissolve the acid substrate. Repeated additions of the enzyme solutions were made at hourly intervals for six hours, after which the reaction mixture was extracted with chloroform. The major product from both enzyme-catalysed reactions was again the keto-imine 279. It was also observed that the product mixture from the laccase-mediated reaction contained a small amount of the 3,4-dihydro-isoquinoline 172 and significantly less polymeric material than the peroxidase induced reaction. No starting material was evident in either reaction mixture. Methylation and reduction failed to show any of the hoped for aporphine 269. A reaction blank containing only substrate in a buffer-ethanol mixture showed only unreacted starting material after standing overnight.

3.3.3 Chemical Oxidations

Compound 279 was found to be the sole product from the oxidation of 245 by active manganese dioxide. Similarly 279 was also the major product from a two phase (chloroform-bicarbonate solution) potassium ferricyanide oxidation, and from oxidation using DDQ. Two minor products from this latter reaction having very similar R_f values to 279 were also detected but could not be separated in sufficiently pure form to be identified. Amido-acid 245 was unaffected by a freshly prepared cuprammonium species.

Although the hydrochloride salt of the 3,4-dihydroisoquinoline 172 was quite stable, the free base form was rapidly converted into the keto-

imine 279 by aerial oxidation. It is therefore not possible to say with any degree of certainty whether 279 arose directly as a result of the anodic, enzymic and chemical oxidations or whether it was formed by aerial oxidation of the initially formed 3,4-dihydroisoquinoline. The overall indications from these various oxidations of acid 245, are that nucleophilic attack of the 1-benzyl residue on a possible quinone methide intermediate structure does not take place, and that the predominant reaction, after or concurrent with decarboxylation, is the removal of the N-blocking group accompanied by oxidation at the benzylic position.

The attempts to prepare an amido-acid analogous to 245, in which the benzylic position is substituted with two methyl groups, thereby preventing oxidation at this susceptible position, is discussed in Chapter 5 (Section 5.2).

CHAPTER FOUR

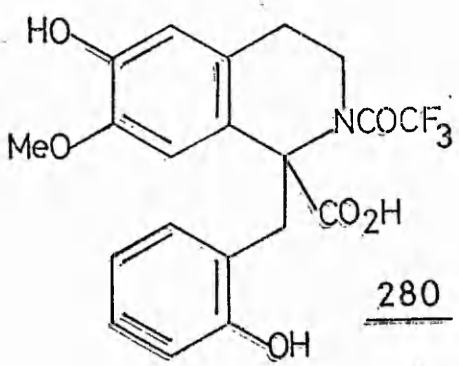
Attempted Synthesis of a Cularine-Type Alkaloid by Oxidative Decarboxylation of an Isoquinoline-1-Carboxylic Acid

Based upon similar arguments to those discussed in the introduction to the previous Chapter (see page 75), oxidative decarboxylation of acid 280 could give rise to the dienone compound 281. Subsequent intramolecular Michael addition of the 2'-hydroxy moiety upon this dienone ought therefore to generate the cularine-type alkaloid 282 by a completely novel approach. The work described in this Chapter was therefore aimed at preparing such a compound both by conventional methods and *via* this decarboxylation-addition pathway.

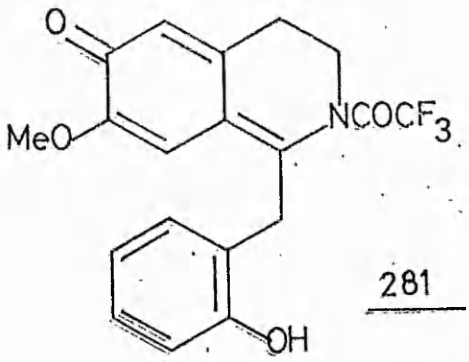
4.1 Synthesis of Cularine Alkaloids

To date only four naturally occurring members of this class of alkaloids have been isolated, viz cularine 283, cularimine 284, cularidine 285 and cularicine 286. These four tetracyclic bases are only found in the genera *Dicentra* and *Corydalis*, and are unusual in that they possess an oxepin ring system, bridging the benzyl and isoquinoline moieties of the molecule. Such a system is considered to be derived from a 7,8-oxygenated precursor e.g. petaline 89. The biosynthetic pathway to such alkaloids has as yet not been elucidated, although several possible routes have been postulated (see Schemes VII-IX).

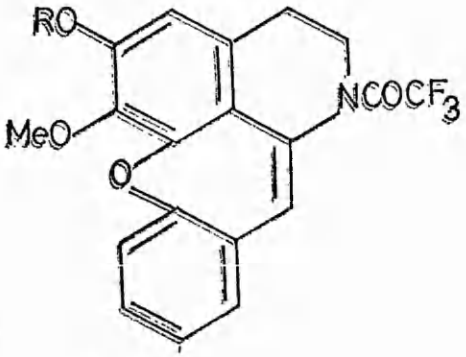
In comparison with other classes of isoquinoline alkaloids, relatively few *in vitro* syntheses of cularine alkaloids have been reported. The first synthesis of cularine 283 involved as the initial step, linking of two appropriately substituted aryl units by an Ullmann condensation, to give a diphenyl ether. This approach has been utilised by Kametani and his co-workers, who have synthesised the cularine ring system, both by a Pomerantz-Fritsch cyclisation of the appropriate acetal[315] and



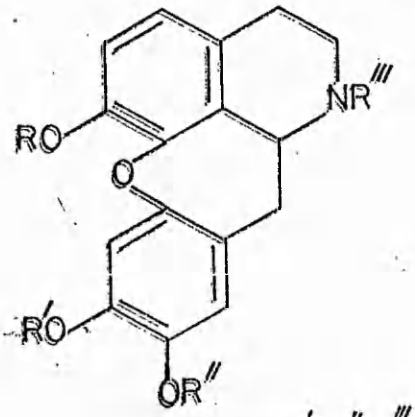
280



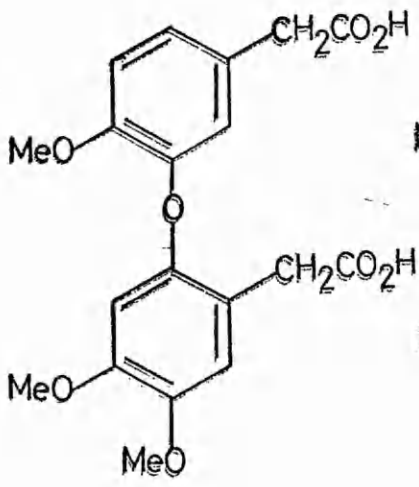
281



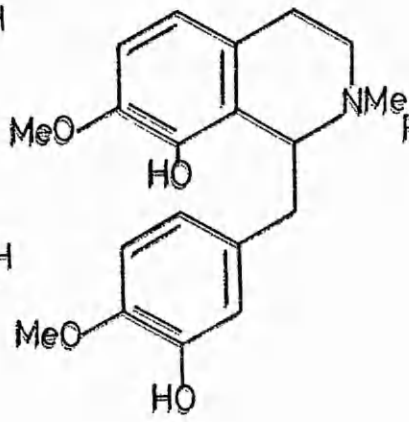
282 R=H
303 R=Me



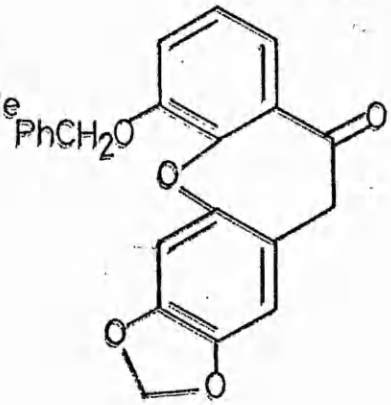
283 R=R′=R″=R‴=Me
284 R=R′=R″=Me, R‴=H
285 R=H, R′=R″=R‴=Me
286 R=H, R′R″=CH_2, R‴=Me



287



289



288

by dehydration of the diacid 287 and subsequent reaction with ammonia.[316,317] Similarly the preparation of cularicine 286 has been achieved[318] through the intermediacy of the tricyclic oxepinone 288 with subsequent Schiffs base formation and a modified Pomerantz-Fritsch cyclisation. The Ullmann ether synthesis has also been used as the final step in the preparation of cularine-type products by cyclising 1-(2'-hydroxybenzyl)-8-bromo-1,2,3,4-tetrahydroisoquinolines.[319,320] An alternative approach involving Ullmann condensation of an isoquinoline having a 2'-brominated D ring and the hydroxy function at C-8 on ring A has also been successfully applied to the synthesis of cularine compounds.[321-5]

One of the proposed schemes for the biosynthesis of these alkaloids involves direct carbon-oxygen coupling of a diphenol benzylisoquinoline (Scheme VII). Such a reaction using potassium ferricyanide has proved successful in the laboratory, giving the appropriate cularine compounds, albeit in low yields. The work performed by Kametani in this respect has been summarised[249,326]. Cularine has been prepared in 7% yield by direct phenol oxidative coupling of 289 using ferricyanide as the oxidant,[327,328] the substrate 289 being prepared via a modified Pomerantz-Fritsch cyclisation with subsequent Reissert alkylation, to give the required substitution pattern on ring D.

An alternative approach involving acid catalysed rearrangement of spirodienone intermediates derived from 4'-hydroxybenzyl isoquinolines has also been successfully applied to the synthesis of certain cularine-type compounds.[102,329] However, cularine itself could not be prepared by such a route, the appropriate dienone giving either phenolic 1-benzylisoquinolines[330,331] or aporphine[278] products, depending upon the nature of the acidic media used for rearrangement.

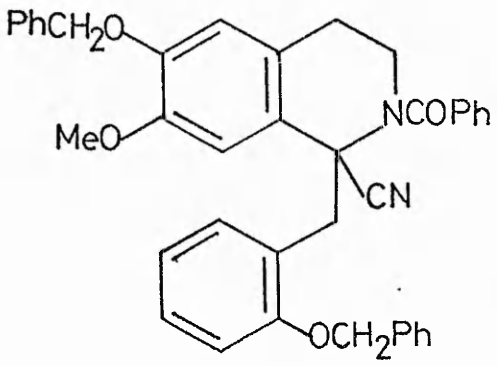
4.2 Synthesis of Substrates

4.2.1 N-Trifluoroacetyl-1-(2'-Hydroxybenzyl)-6-Hydroxy-7-Methoxy-1,2,3,4-Tetrahydroisoquinoline-1-Carboxylic Acid 280

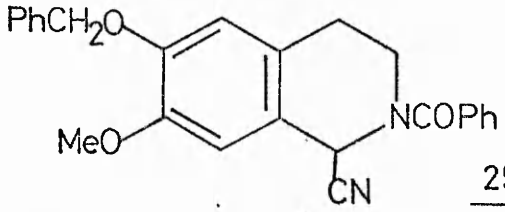
Two independent approaches to the N-acylated acid 280 were investigated. The first involved the preparation and acid hydrolysis of the appropriately substituted Reissert compound 290, whereas the second route required a Pictet-Spengler condensation between the appropriate phenylethylamine and substituted pyruvic acid.

The novel dihydro-Reissert compound N-benzoyl-1-cyano-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 291 was prepared in 60% yield from the 3,4-dihydroisoquinoline 292 using the two phase method similar to that used for the isomeric compound 186. Addition of 291 into a suspension of sodium hydride in DMF produced the expected red colouration of the Reissert anion, which disappeared upon the introduction of a solution of crude 2'-benzyloxybenzyl chloride 293 (prepared from the appropriate benzaldehyde 294 and primary alcohol 295). The product from this reaction could not be sufficiently purified for identification and coupled with other failures to hydrolyse 7-benzyloxy-Reissert compounds to their respective phenolic amino-acids (see page 67), this approach was not continued.

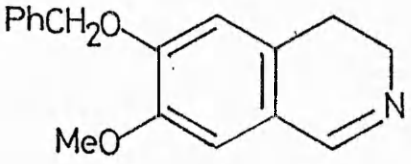
A more successful pathway to the required isoquinoline-1-carboxylic acid involved the Pictet-Spengler condensation between 3-hydroxy-4-methoxy-β-phenylethylamine hydrochloride and 2-benzyloxyphenylpyruvic acid 296, giving the desired novel acid 297 in 90% yield. Preparation of the necessary pyruvic acid 296 was achieved in 57% yield by alkaline hydrolysis of 5-(2'-benzyloxybenzylidene)-2-thioxizolid-4-one 298. Higher yields of this same acid were obtained from hydrolysis of the



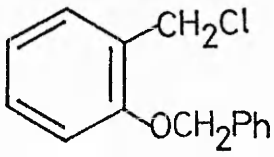
290



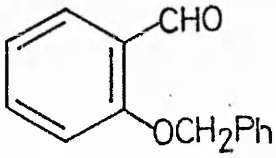
291



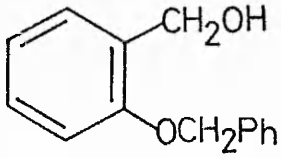
292



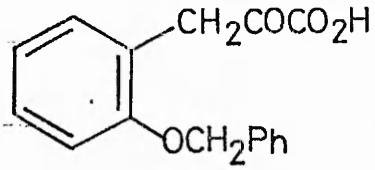
293



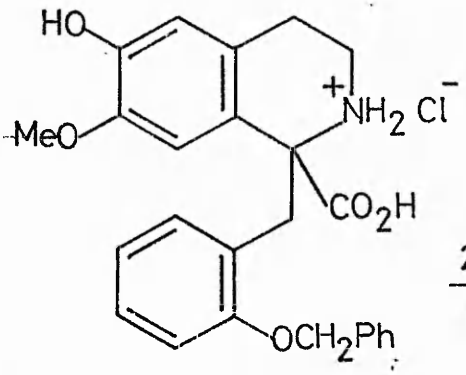
294



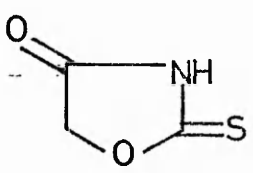
295



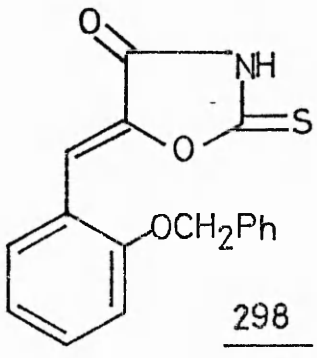
296



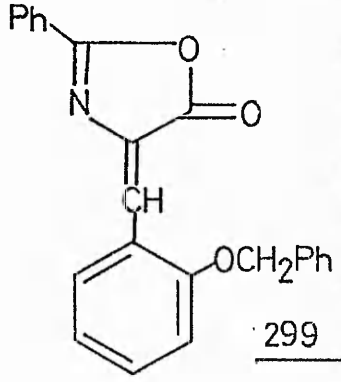
297



355



298



299

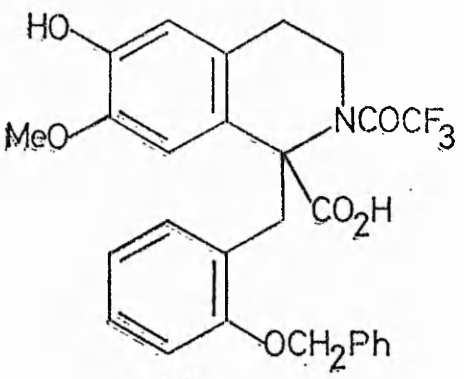
azlactone 299 and purification through the formation of the barium salt.[332] Due to its instability to light and air this product was stored under nitrogen at 4° until required. Although the spectral data for 297 were consistent with this structure, elemental carbon analysis of the hydrochloride salt did not agree with the expected values.

N-Trifluoroacetylation of 297 using trifluoroacetic anhydride in chloroform at room temperature gave the 2'-benzyloxy acid 300 in 47% yield. Earlier attempts to prepare either the N-formyl, N-acetyl and N-ethoxycarbonyl analogues of 300 using acetic-formic anhydride,[310] acetyl chloride[1] or ethyl chloroformate respectively, were unsuccessful. Similarly, attempts to acetylate on nitrogen using α,α-dicyanoethyl acetate[333] were also fruitless. However reaction of acetic-formic anhydride with the novel debenzylated acid 301 gave a small amount of the δ-lactone 302. Spectral data and elemental analysis confirmed the lactone structure rather than the 2'-hydroxy-acid alternative.

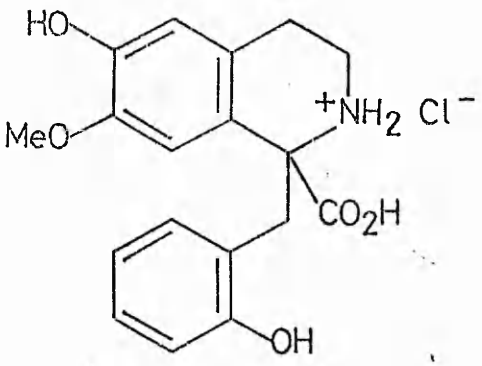
In view of the relative ease of use and good yields obtained with trifluoroacetic anhydride, the N-trifluoroacetylated system was chosen in preference to N-formyl analogues. Several attempts to prepare the 2'-hydroxy compound 280 by catalytic hydrogenolysis of 300 with 5% palladised charcoal failed to produce the expected results, for which the most likely explanation was contamination of the palladium catalyst. Unfortunately lack of time prevented an alternative synthesis of 280, involving N-trifluoro acetylation of the dihydroxy acid 301, from being investigated.

4.2.2 Attempted Synthesis of the Dehydrocularine Compound 303

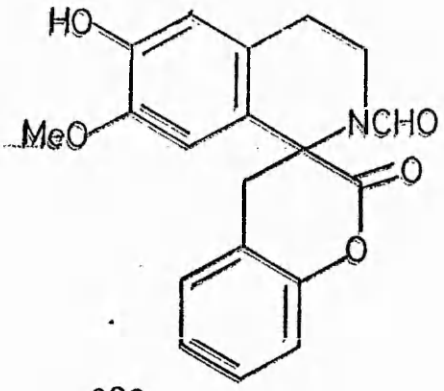
Several different synthetic routes to the required dehydrocularine



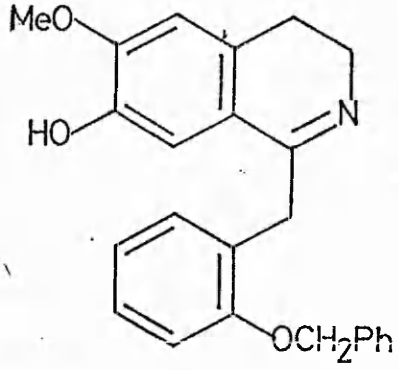
300



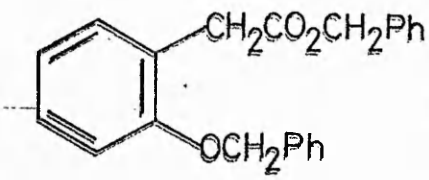
301



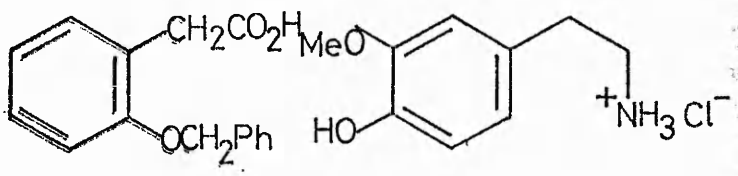
302



304

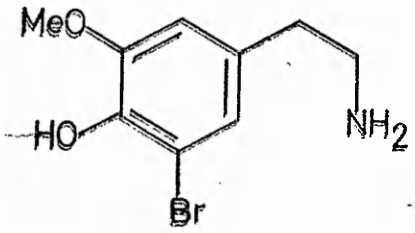


305

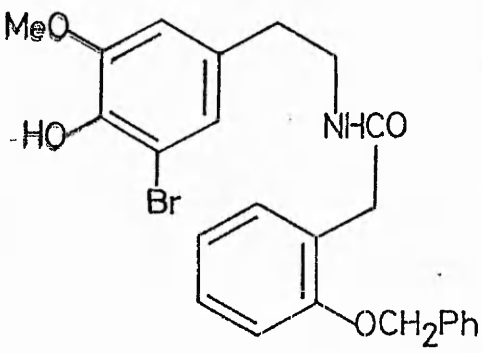


306

307



308



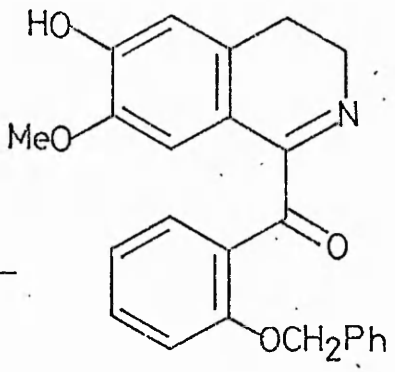
309

structure 303 were followed, all involving at some stage, a Bischler-Napieralski reaction, to generate the isoquinoline ring system. To effect the final ring closure of a suitably substituted 1-benzylisoquinoline to the corresponding oxepin system, it was intended to use pentafluorophenyl copper, which has been used[334] to bring about intermolecular Ullmann condensation in relatively high yields.

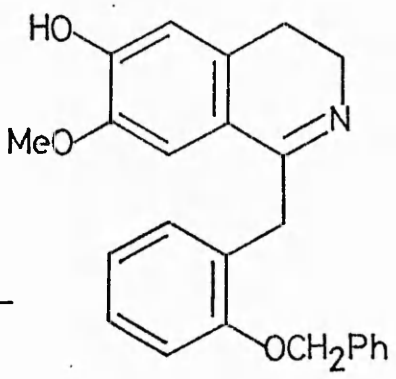
The first proposed synthesis required the formation of 3,4-dihydroisoquinoline 304 followed by monobromination at the C-8 position,[335] with subsequent debenzylation and cyclisation. The major product from the reaction of 2-hydroxyphenylacetic acid and benzyl chloride was found to be the benzyl ester 305, which gave the desired 2-benzyloxy acid 306 in 62% yield upon alkaline hydrolysis. The attempted condensation of this acid with 3-methoxy-4-hydroxy-β-phenylethylamine 307 according to the method reported by Casagrande[288] failed to give the required phenolic amide, giving only an intractable gum. Using the same experimental conditions, condensation of 2-benzyloxyphenylacetic acid and 3-methoxy-4-hydroxy-5-bromo-β-phenylethylamine 308 (prepared by bromination[336] of 307) furnished 31% of the brominated amide 309. Unfortunately attempts to cyclise this amide using phosphorus oxychloride in acetonitrile[227] were unsuccessful.

An alternative approach to the required dehydrocularine alkaloid via the dihydroisoquinoline 310 was next investigated. This route involved the synthesis of 3-benzyloxy-4,5-dimethoxy-β-phenylethylamine from gallic acid according to Scheme XXI. Preparation of the phenolic acid 311 according to Battersby's[337] method gave only 7% of the expected compound, the major product being 3,4,5-trimethoxybenzoic acid. Benzylation of 311 was achieved using benzyl chloride in alkaline

310

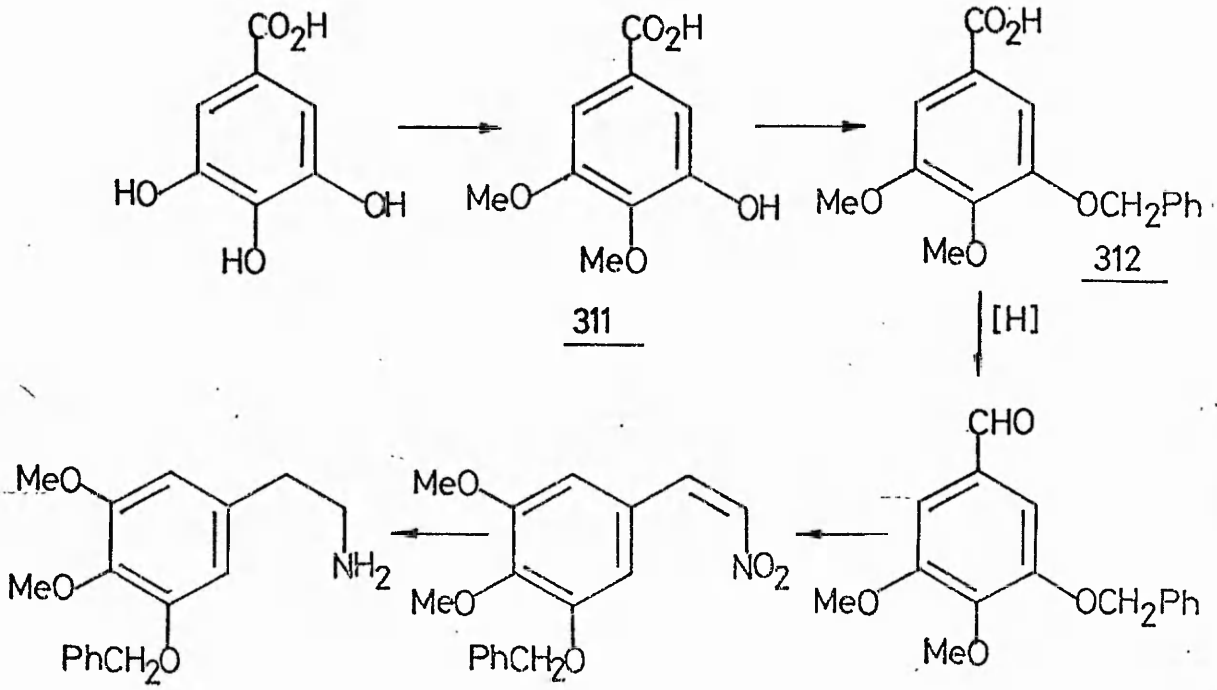


313



314

SCHEME XXI



311

312

ethanol, to give 3-benzyloxy-4,5-dimethoxybenzoic acid 312 in only 22% yield. In view of low yields achieved in these two steps this route was not pursued further.

4.2.3 Oxidation of Acid 300

In spite of the failure to produce acid 280 from its benzyloxy precursor 300, it was hoped that useful information could be gained by oxidising this precursor both electrochemically and enzymically. Anodic oxidation of 300 on a carbon felt electrode in an aqueous methanolic solution of sodium bicarbonate was carried out at an applied potential of 280 mV (with respect to the standard calomel electrode). The product obtained from this reaction was identified as the keto-imine 1-(2'-benzyloxybenzoyl)-6-hydroxy-7-methoxy-3,4-dihydroisoquinoline 313. This same compound was shown to be the only product formed by the oxidation of 300 using both horseradish peroxidase and fungal laccase. The possibility also exists, as with the 3,4,5-trimethoxy-analogue 279 that 313 could be formed by initial deacylation and oxidative decarboxylation to give the 3,4-dihydroisoquinoline 314 which undergoes rapid aerial oxidation at the susceptible benzylic position.

CHAPTER FIVE

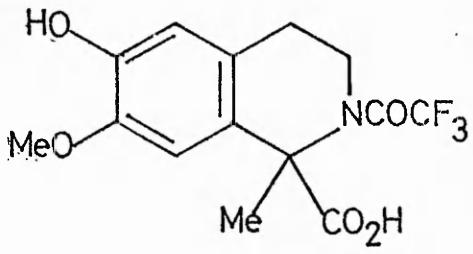
Discussion

This chapter includes several sections of related work which were investigated during the course of this thesis, but which do not warrant individual chapters.

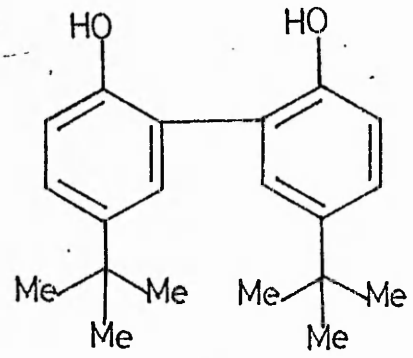
5.1 Oxidative Studies on N-Trifluoroacetyl-1-Methyl-6-Hydroxy-7-Methoxy-1,2,3,4-Tetrahydroisoquinoline-1-Carboxylic Acid 315

The work described in Chapters 3 and 4 was concerned with the oxidative decarboxylation of phenolic isoquinoline-1-carboxylic acids to a dienone intermediate which, it was considered, could in the presence of a suitably activated 1-benzyl residue undergo intramolecular attack to give tetracyclic products. In addition to this intramolecular coupling mode, the possibility also exists that in the presence of an external nucleophilic species, any dienone produced could be involved in intermolecular coupling. The object of this section of work was therefore to investigate the possible occurrence of such an intermolecular reaction between N-trifluoroacetyl-1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 315 and a suitable nucleophile. N-trifluoroacetylation of acid 164 was carried out in the previously reported manner using trifluoroacetic anhydride in chloroform and triethylamine to give 315 in 52% yield.

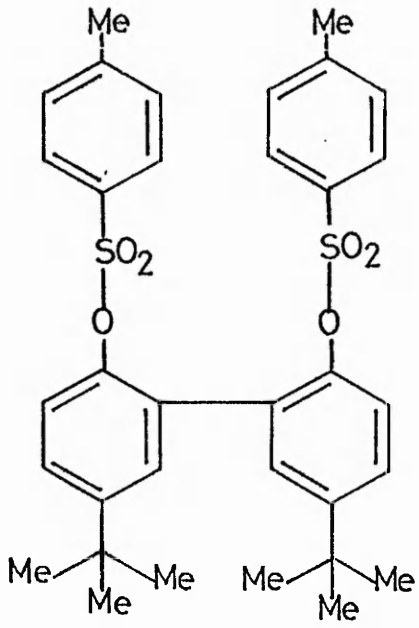
The nucleophile chosen was 4-tert-butylphenol because it was thought that should any intermolecularly linked product be formed, then it ought to be easily identifiable by the distinctive nmr signal of the tert-butyl group. Since phenols themselves readily undergo oxidation it was also decided to prepare the carbon-carbon dimer 316 of 4-tert-butylphenol, so that this particular product could be identified in the oxidation


HO
MeO
NCOCF3
Me
CO2H

315


HO
HO
Me
Me
Me
Me
Me
Me

316


Me
Me
SO2
SO2
O
O
Me
Me
Me
Me
Me
Me

317

mixtures. Dimer[338] 316 was synthesised by the electrochemical oxidation of 4-tert-butylphenol in its sodium salt form, using a carbon felt electrode and an applied potential of 300mV in acetonitrile. In order to confirm that it was in fact the carbon-carbon rather than the carbon-oxygen-carbon dimer, the product from the anodic oxidation was tosylated; the nmr showing the correct ratio of methyl to tert-butyl protons, consistent with the ditosyl product 317.

Oxidation of acid 315 was performed electrochemically and enzymically, both on its own and in the presence of 4-tert-butylphenol. Anodic oxidation in aqueous methanolic sodium bicarbonate solution at 320mV showed the same results irrespective of whether the phenol was present or not, the only product being identified as the 3,4-dihydroisoquinoline 170. Similarly, enzymatic oxidations of 315 with fungal laccase and horseradish peroxidase showed 170 as the major component, both with and without added 4-tert-butylphenol, in addition to which some polymeric base line material was observed. In general the horseradish peroxidase reaction media contained substantially more base line material than their laccase counterparts, probably a consequence of the free-radical pathway associated with peroxidase-peroxide combinations. None of the phenolic dimer 316 was apparent in either anodic or enzymic reaction mixtures containing 4-tert-butylphenol.

The indications from this experiment are that the N-protected acid 315 is both deacylated and oxidatively decarboxylated, although in which order it is not possible to say. Furthermore, intermolecular coupling between the decarboxylated intermediate and some external nucleophile does not take place, under the reaction conditions employed.

5.2 Attempted Synthesis of β,β-Dimethyl-3,4,5-Trimethoxyphenylpyruvic Acid 318

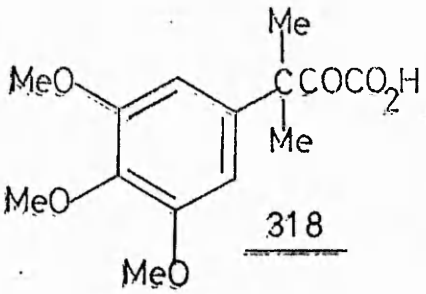
In view of the susceptibility of the benzylic position of N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 245 to undergo oxidation, giving the corresponding keto compound 279, the possibility of replacing both of the labile benzylic hydrogen atoms by less easily removed alkyl groups was investigated. The use of methyl groups for this purpose was decided upon, since such groups would minimise the increase in steric strain in an already crowded environment; it was thus necessary to prepare the appropriately substituted α-keto acid 318, having two β-methyl substituents. Condensation of this pyruvic acid with 3-hydroxy-4-methoxy-β-phenylethylamine hydrochloride, would it was hoped, give an isoquinoline-1-carboxylic acid having a benzylic position reasonably resistant to oxidation, enabling the true products from the anodic, enzymic and chemical oxidation studies to be identified, without the complication of subsequent aerial oxidation.

The proposed synthetic pathway to the β-protected α-keto acid 318 is shown in Scheme XXII. The final stage of this sequence involves oxidation of a ketone to an α-keto acid by selenium dioxide; a similar reaction has been carried out in very high yield by Hallmann and Hagele.[339] α,α-Dimethyl-3,4,5-trimethoxybenzyl alcohol 319 was best prepared by the reaction of the methyl ester 320 with methyl lithium in dry ether, giving 319 in 85% yield. The molarity of the lithium reagent was determined before each reaction according to the method of Kofron.[340] Other methods of preparing 319 were found to be less successful. Only 51% of tertiary alcohol was obtained from 3,4,5-trimethoxyacetophenone 321 using an identical procedure to the above; this result was

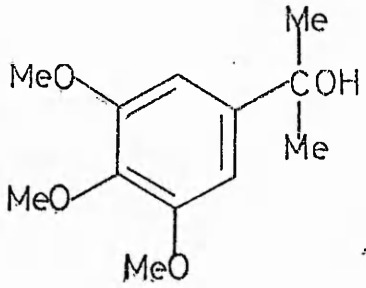
disappointing since the first step of the reaction of methyl lithium with ester 320 involves the preparation of this same ketone,[341] followed by the addition of a further mole of methyl lithium to give alcohol 319. The yield of 319 by this method was therefore expected to be at least the same as that from the ester, at the same time using less alkyl lithium reagent. Reaction of ester 320 or the acid chloride 322 with methyl magnesium iodide gave the tertiary alcohol in unacceptably low yields and often associated with other components; this is similar to results reported by Bogert and Isham.[376]

Attempts to convert 319 into its chloro- or bromo- analogue by using phosphorus tribromide, hydrochloric acid, triphenylphosphine in carbon tetrachloride[343] or a mixture of methyl sulphide and N-chlorosuccinimide[344] gave only 3,4,5-trimethoxy-α-methylstyrene[342] 323. An alternative approach involved the synthesis of methyl α,α-dimethyl-3,4,5-trimethoxybenzylsulphonate 324. Nucleophilic displacement of the mesyl group by a halogen species ought to produce the required benzyl halide; however, although the methyl sulphonate 324 was prepared, albeit in low yield from alcohol 319 and mesyl chloride in dry pyridine, all subsequent attempts to prepare this compound resulted in the formation of the elimination product 323.

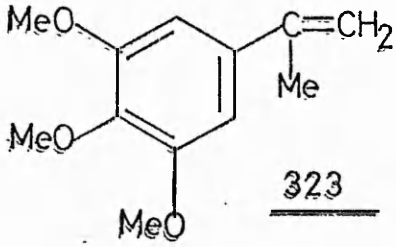
The overlying trend from the observations made in this section of the work is that although the α,α-dimethyl alcohol 319 is stable and can be prepared in high yield, elimination of water to give the corresponding styrene 323 takes place under very mild conditions.



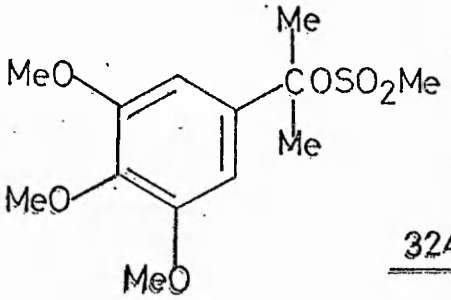
318



319

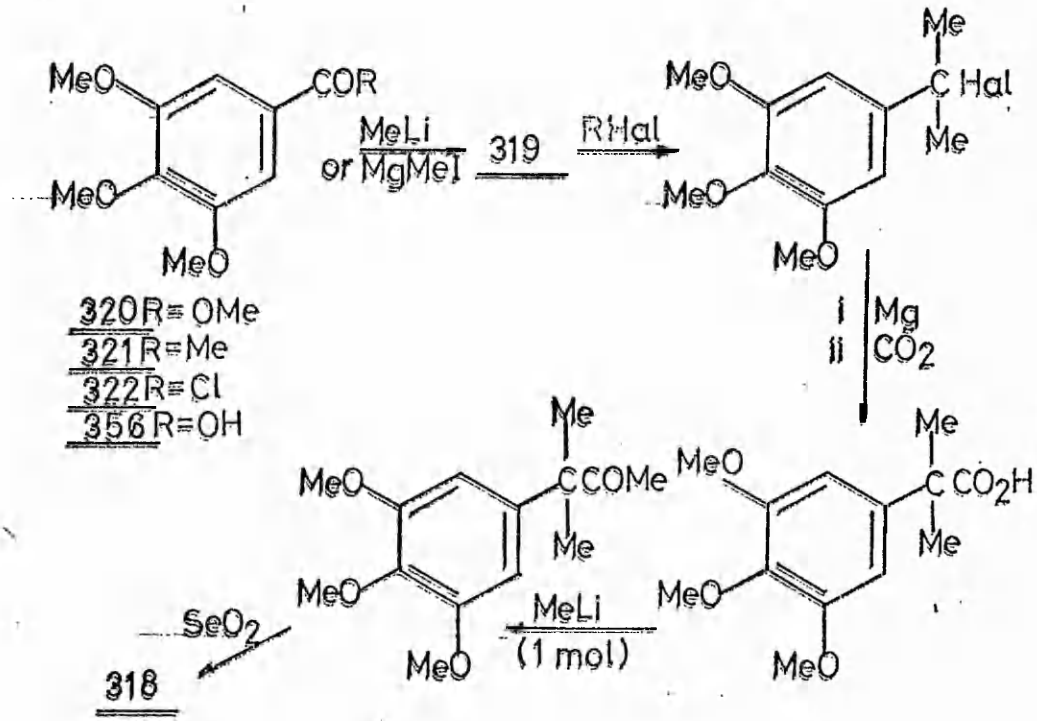


323



324

SCHEME XXII



5.3 Anomalous Product from the Reaction of Amino-Acid 166 and Trifluoroacetic Anhydride in Pyridine

Initial attempts to prepare the N-trifluoroacetylated acid 245 were carried out in refluxing dry pyridine. However, immediately upon boiling, the solution turned intensely yellow, from which homogeneous bright yellow crystals were obtained on a weight for weight basis. The product did not react with saturated bicarbonate solution indicating that the carboxyl grouping had either been removed completely or had been modified into a non-acidic function. Refluxing acid 166 in pyridine gave only unreacted acid, confirming that trifluoroacetic anhydride was essential for the occurrence of this anomalous reaction. High resolution mass spectral data indicated a molecular formula for the product of $C_{24}H_{21}F_6NO_6$, which was confirmed by elemental analysis. The infrared spectrum of this anomalous product showed a broad absorption, centred at $3200cm^{-1}$, indicating that the hydroxyl group was still present in the molecule; this fact was confirmed by the bathochromic shift in the u.v. in basic media, and from nmr data. No other absorptions were apparent in the infrared above $1600cm^{-1}$, from which it was thought that a trifluoroacetyl grouping was absent in the molecule. Proton nmr data showed three singlets corresponding to one proton apiece in the aromatic region, a broad singlet exchangeable with deuterium oxide at 3.72τ, indicative of a phenolic function, a quartet at 4.50τ having a coupling constant of 7.5Hz, integrating for a single proton and four distinct singlets around 6τ corresponding to the four methoxy substituents.

Several conclusions can be made from the above nmr information; firstly, the aromatic substitution pattern appeared to be unchanged, having one hydroxyl and four methoxy groups; secondly, since only three protons were observed in the aromatic region then either aromatic substitution

had occurred, or as seems more likely, ring closure had taken place; finally, the occurrence of the low field quartet can only be due to coupling between a single proton and three other magnetically active, equivalent nuclei, the most probable explanation being coupling with a trifluoromethyl group, i.e. a $CH-CF_3$ unit. The presence of this particular arrangement was confirmed by the ^{19}F nmr, which exhibited a doublet at 75.47ppm (w.r.t. $CFCl_3$ standard) having a coupling constant virtually identical to that observed in the proton nmr. In addition, an absorption corresponding to three fluorine atoms was also observed at 70.74 ppm, indicating that another trifluoromethyl group was present in an entirely different environment.

Attempts to acylate the nitrogen atom with trifluoroacetic anhydride in chloroform using either triethylamine or 1,8-bis-(dimethylamino) naphthalene as base were unsuccessful, suggesting that the nitrogen was of a non-nucleophilic nature. However, t.l.c. of the reaction mixture showed the presence of a new component, the infrared spectrum of which had a sharp absorption at $1810cm^{-1}$ and another at $1645cm^{-1}$. Isolation of this compound by chromatographic methods was not possible, giving only the unknown starting material. In view of previous experiences with this sort of phenomenon, e.g. 261 (see page 96), this unobtainable compound was identified as an O-trifluoroacetate formed between the anhydride and the phenolic moiety. The attempted reduction of the yellow compound with either sodium borohydride or catalytically with palladised charcoal had no effect, whereas bromination with excess bromine gave a complex mixture of products.

Methylation of the phenolic unknown compound was achieved both with diazomethane and with dimethyl sulphate, although the latter was less

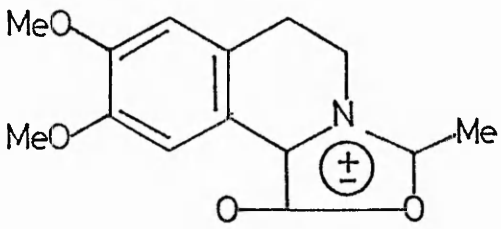
efficient. The methylated product showed similar spectroscopic features to those of its precursor, having a molecular formula of $C_{25}H_{23}F_6NO_6$. Monomethylation had therefore taken place and since the hydroxy stretch in the infrared, bathochromic shift with base in the u.v. and the exchangeable proton in the nmr spectrum, were no longer apparent, it was obvious that the phenolic group had been converted into a methyl ether. An extra methoxy signal in the nmr and an absorption at 1633cm^{-1} in the infrared were also observed. Efforts to quarternise the nitrogen atom of this fully methylated analogue with methyl iodide were unsuccessful, as was the attempted reduction of the molecule with tin and hydrochloric acid, only intractable products being obtained.

It was initially postulated that the unknown product might be a 6a,7-dehydroaporphine, since molecules of this type are known to be yellow,[122] and would have two aromatic protons and one vinylic proton, in agreement with the observed nmr spectrum. However such a structure could not account for the tertiary nature of the nitrogen atom and the incorporation of six fluorine atoms. Another possible explanation was a structure similar to the munchnones reported by Hershenson,[345] who proposed that the reaction of N-acetyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid with acetic anhydride in pyridine involved the formation of the mesoionic compound 325 (a munchnone). Similarly Steglich and his co-workers[346] have employed trifluoroacetic anhydride to convert α-amino acids into oxazolin-5-ones containing a $CH\text{-}CF_3$ unit. Neither of these two structures is capable of explaining all of the experimental information.

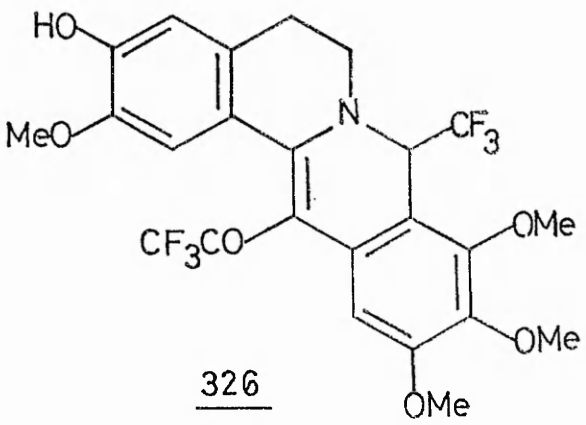
Identification of the unknown product and its methyl ether was achieved in collaboration with Drs. Boyle, Greenbanks and Taylor of I.C.I.

Pharmaceuticals Division, Alderley Park, for which the author wishes to express his gratitude. The initial product was thought to be 2,9,10,11-tetramethoxy-3-hydroxy-8-trifluoromethyl-13-trifluoroacetyl-7,8-dihydro-protoberberine 326, which on methylation results in the formation of the novel methyl ether analogue 327. Compounds possessing the N-C=C-C=O arrangement have been termed enaminones[347] and the unusual spectroscopic characteristics of such compounds have recently been investigated. Taylor[348,349] found that both the carbonyl and double bond infrared absorption frequencies of enaminones (also called vinylogous amides) occurred at much lower wavenumbers than for identical functions in isolated environments. This effect was found to be more pronounced when the heteroatom and keto group were trans to each other, and in general it was found that the C=O frequency was lowered by 65cm^{-1} whereas the C=C was usually 85cm^{-1} lower than typical values. Application of these findings to compound 327 shows good agreement between the predicted and found νC=O and νC=C, (i.e. found 1633cm^{-1} and 1585cm^{-1}, calculated 1635cm^{-1} and 1575cm^{-1}, assuming isolated absorption values of 1700cm^{-1} for N-trifluoroacetyl and 1660cm^{-1} for carbon-carbon double bond). In the case of 326 the phenolic function is in extended conjugation with the enaminone system, resulting in a further lowering of absorption frequencies to 1595cm^{-1} and 1550cm^{-1}. In polar solvents trans enaminones have been observed[347] to give u.v. maxima between 285-305nm, compared to maxima between 300-320nm when in the cis configuration. Both 326 and 327 gave u.v. absorption maxima in agreement with a trans arrangement.

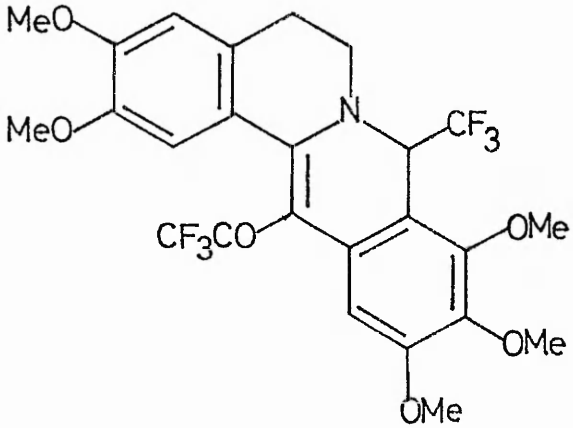
Although a mechanism can at this stage only be pure conjecture, a possible route to 326 is shown in Scheme XXIII. Additional work is therefore necessary to establish this or any other possible mechanism. In view of the novelty of this reaction, several variations are worthy


MeO
MeO
N
Me
O
O

325

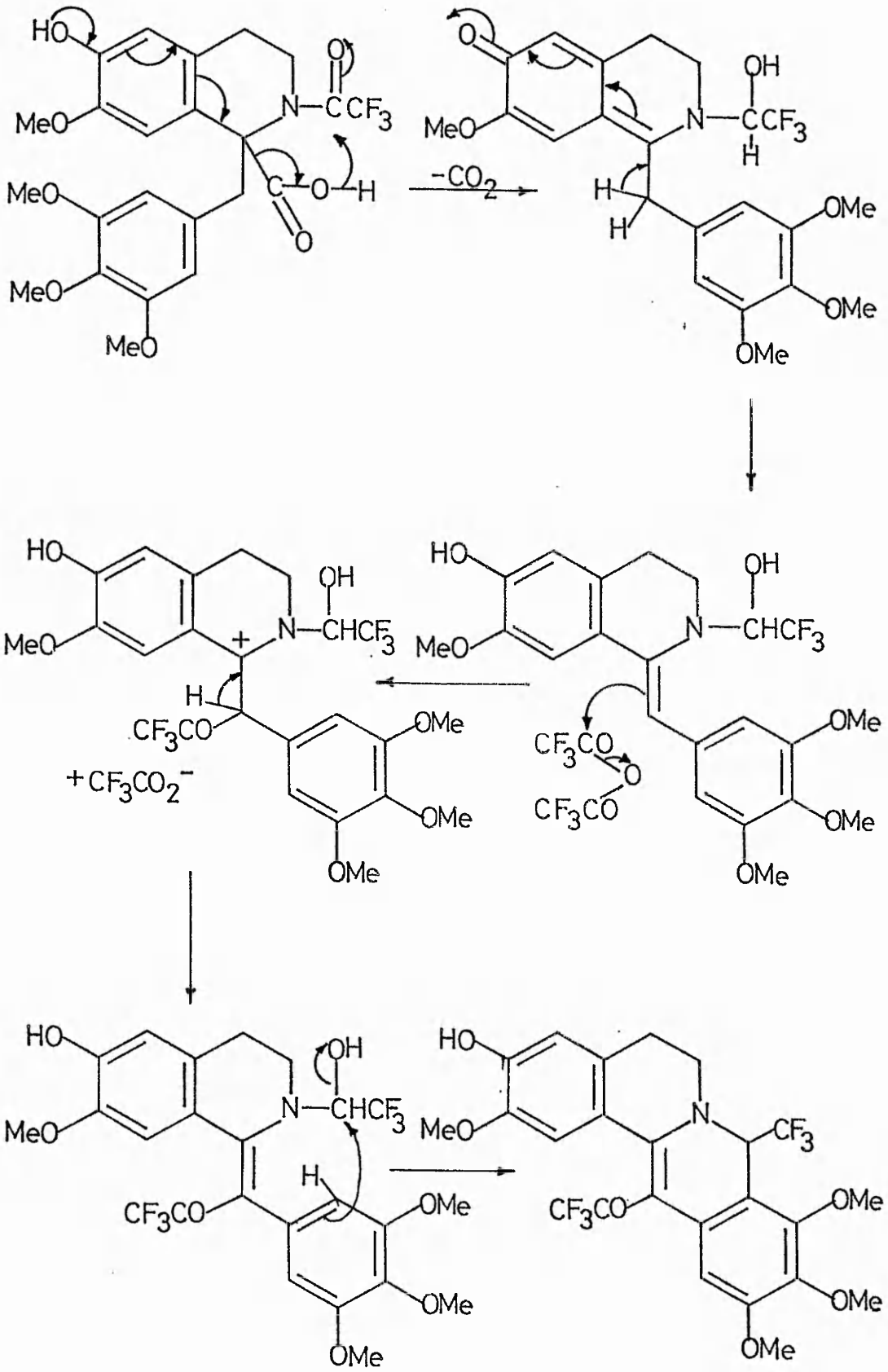

HO
MeO
N
CF3
CF3CO
OMe
OMe
OMe

326


MeO
MeO
N
CF3
CF3CO
OMe
OMe
OMe

327

SCHEME XXIII



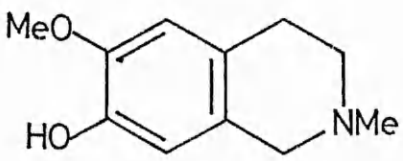
of further investigation to establish the extent to which such a reaction can be extended, e.g. by changing the methoxylation pattern on ring D; replacing the methoxy substituents on this same ring by other oxygenated groups (e.g. methylenedioxy); establishing the requirement for a phenolic function at C-6; investigating the effect of other anhydrides (e.g. acetic and trichloroacetic anhydride).

5.4 Enzymic Oxidation of Corypalline 328

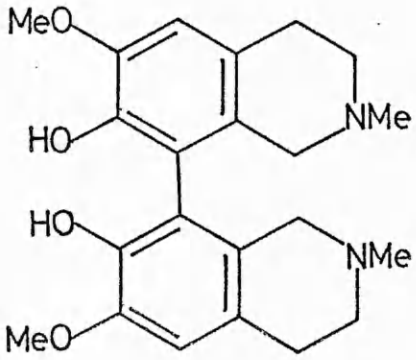
Corypalline (N-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline) 328 is a naturally occurring simple isoquinoline alkaloid, first identified by Manske[350] and shown to contain the C-6, C-7 oxygenation pattern found in more complex alkaloids.

Several workers have studied the oxidation of 328 by various methods. Bobbitt, in an extensive study[351-353] has shown that the electrochemical oxidation of corypalline produces N,N′-dimethyl-6,6′-dimethoxy-7,7′-dihydroxy-1,1′,2,2′,3,3′,4,4′-octahydro-8,8′-diisoquinoline 329 as the major product, in yields up to 85%, depending upon the nature of the electrodes, electrolyte, cell design, pH, reaction time and the state of the substrate. Oxidation of the sodium salt was achieved at much lower potentials than its free phenolic form and subsequently resulted in less polymeric material as a result of over-oxidation. Small amounts (2.7-5%) of the carbon-oxygen-carbon dimer 330 have also been reported *via* anodic oxidations. In contrast, the catalytic oxygenation of corypalline using platinum oxide gave rise to other products, viz, the polycyclic ether 331, the 3,4-dihydroisoquinolinium and isoquinolinium salts 332 and 333, in addition to those products derived electrochemically; however, the yields of these other compounds were low (2-4%). Chemical oxidation of 328 using potassium ferricyanide[354,355] gave 329 in moderate yields, whilst photochemical oxidation of corypalline produced 329 in 33% yield.

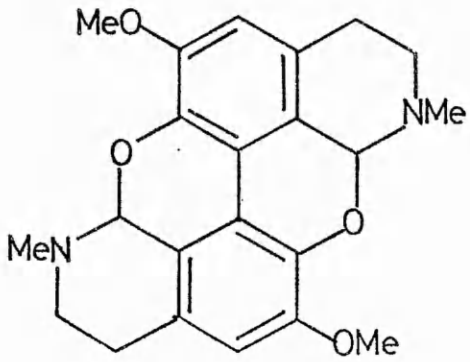
The carbon-carbon dimer is the expected product, based upon steric considerations and it has been demonstrated[352] that the larger the C-1 substituent the greater the extent of the carbon-oxygen-carbon product, resulting from an increased steric hindrance about the C-8, C-8′ bond.



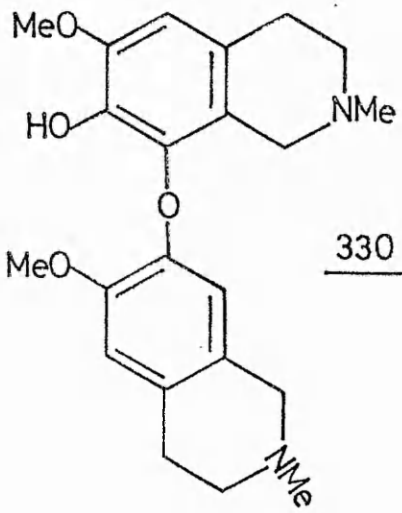
328



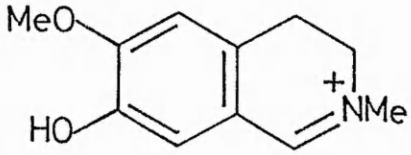
329



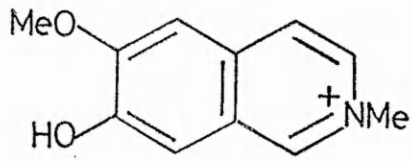
331



330



332



333

At the time of writing, no investigation into the effect of oxidising enzymes on 328 had been reported. A study was therefore carried out using the phenol oxidising enzymes, fungal laccase and horseradish peroxidase under physiological conditions. A sample of corypalline was obtained from Dr. I. Coutts. Electrochemical oxidation of 328 as its sodium salt at 0.0mV (w.r.t. the standard calomel electrode) in methanol using a carbon felt electrode furnished 22% of dimer 329. This dimeric product was used as a t.l.c. marker spot throughout the enzyme study.

Incubation of corypalline with both fungal laccase and peroxidase-peroxide in pH6 aqueous media showed the formation of 329 after 21 hr. No other components other than unreacted starting material were observed. It was therefore concluded that both enzymes used are able to induce ortho-ortho phenol oxidative coupling in corypalline.

EXPERIMENTAL SECTION

General

Infrared spectra were recorded using either a Perkin Elmer 137 NaCl, or a Perkin Elmer 137G grating spectrophotometer both calibrated with polystyrene film. Ultra-violet absorption spectra and absorbance measurements were determined using a Perkin Elmer 402 UV-visible spectrophotometer.

Proton magnetic resonance spectra were recorded on a JEOL JNM C-60 HL 60MHz spectrophotometer with tetramethylsilane as the internal standard in the solvent indicated. ^{19}F Nuclear magnetic resonance spectra were carried out in the Department of Chemistry, University of Nottingham. ^{13}C and 220MHz proton, nuclear magnetic resonance spectra were recorded by the Physico-Chemical Measurements Unit, Harwell.

Melting points were determined using open capillary tubes in an electrically heated Gallenkamp melting point apparatus and are corrected.

Micro analyses for C,H,N and Br were determined by the micro analysis unit, University of Nottingham, by Butterworths Microanalytical Consultancy Ltd., Teddington, Middlesex and by the analytical section of Imperial Chemical Industries Ltd., Alderley Edge, Macclesfield. Analyses for F were carried out at the Department of Chemistry, University of Durham.

High and low resolution mass spectrometry determinations were carried out by the Boots Co. Ltd., Nottingham. Hydrogenations were performed using the medium pressure apparatus of Chas. W. Cook and Sons, Birmingham, while a Hanovia 1L(90watt) photochemical reactor was employed for the photolytic work. Electrochemical experiments were

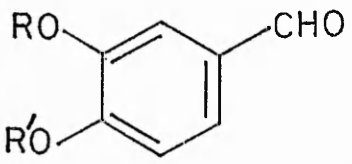
performed on a Wenking potentiostat model 70TS1 using a specified reference electrode.

Thin-layer chromatography was carried out using pre-spread plates (5 x 20cm; Polygram SIL G/UV_{254} and Polygram ALOX N/UV_{254} from Camlab, Cambridge). Preparative t.l.c. (thick layer) was performed on pre-spread plates (20 x 20cm; Anachem Uniplate, silica-gel G.F.). Column chromatography was carried out using Fison silica-gel MFC (80-200 mesh) and Fison alumina (100-250 mesh).

All solvents for chromatographic and photolytic work were redistilled. Ether, THF and benzene were dried over sodium, methanol was dried using magnesium, and chlorinated solvents were dried over calcium chloride. Other solvents were dried using type 5A molecular sieves.

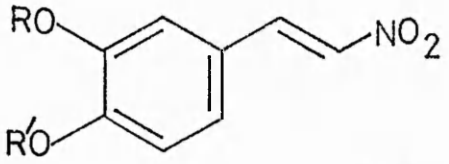
General anodic oxidation procedure

All anodic oxidations were carried out on a carbon felt electrode (6 x 16cm), separated from a platinum cathode (1.5 x 1.5cm) by a porous glass frit. The reactions were performed under nitrogen at room temperature. A standard calomel reference electrode was positioned as close as possible to the anode and the potential controlled electronically. Substrates were added to a stirred pre-equilibrated, pre-electrolysed deoxygenated solvent. The anode potential was adjusted so that 20-50mA of current passed, reactions being continued until either the current dropped back to the residual background level or until t.l.c. indicated no starting material remained.



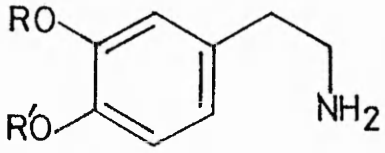
334 R=$PhCH_2$, R′=Me

348 R=Me, R′=$PhCH_2$



335 R=$PhCH_2$, R′=Me

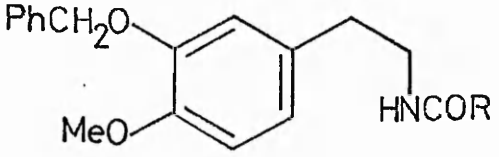
349 R=Me, R′=$PhCH_2$



336 R=$PhCH_2$, R′=Me

337 R=H, R′=Me

347 R=Me, R′=$PhCH_2$

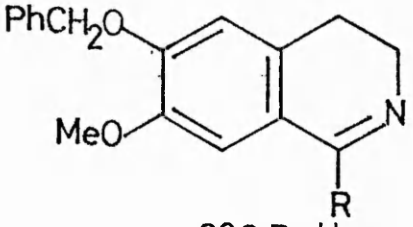


338 R=H

339 R=Me

341 R=$PhCH_2$

272 R=3,4,5$(MeO)_3PhCH_2$

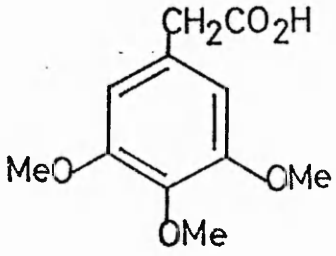


292 R=H

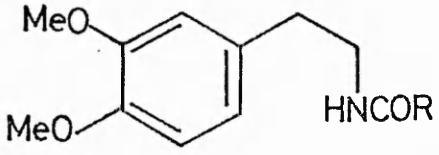
340 R=Me

342 R=$PhCH_2$

273 R=3,4,5$(MeO)_3PhCH_2$



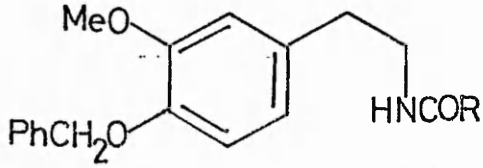
343



344 R=H

345 R=Me

346 R=$PhCH_2$



350 R=H

351 R=Me

352 R=$PhCH_2$

WORK DESCRIBED IN CHAPTER TWO

Preparation of Fungal Laccase

Growth of Fungus

A culture of Polyporus versicolor, strain 28A, growing on an agar slant was obtained from the Building Research Establishment, Princes Risborough, Aylesbury, Bucks. The organism was sub-cultured on to agar plates and grown at 30°. The liquid growing medium[175] was prepared from the following A.R. grade chemicals:-

glucose	20g
L-asparagine	2.5g
DL-phenylalanine	0.15g
adenine	0.0275g
thiamine hydrochloride	0.00005g
potassium dihydrogen phosphate	1.0g
disodium hydrogen phosphate $2H_2O$	0.1g
magnesium sulphate $7H_2O$	0.5g
calcium chloride	0.01g
ferrous sulphate $7H_2O$	0.01g
manganous sulphate $4H_2O$	0.001g
zinc sulphate $7H_2O$	0.001g
copper sulphate $5H_2O$	0.002g

dissolved in distilled water ($1dm^3$).

The medium was equally distributed into five conical flasks ($250cm^3$) which were sterilised by autoclaving (120° for 30 mins.). A single plate of freshly grown fungus was sub-divided into five sections of approximately equal areas, one piece being added to each conical flask under sterile conditions. The flasks were incubated at 25° for 7 days at which point a solution of 2,5-dimethylaniline in 50% aqueous ethanol ($0.4cm^3$ of 0.2M solution) was added to each flask to give a final concentration of the aniline in each flask of $4 \times 10^{-4}M$. After an additional 7 days incubation at 25° the cultures were harvested. Glass beads (100g) were added to each flask which was shaken manually for 10 mins. to break-up the mycelial growth. The resulting suspension

was filtered through Whatman No. 1 filter paper and the filtrate stored at -20° in clean polythene bottles.

Assay of Laccase Activity

Assays were carried out using the method of Pickard and Westlake[223]. Assay mixtures contained catechol (1×10^{-6}mol), acetate buffer (pH4.0, 5×10^{-5}mol) and enzyme solution ($0.1cm^3$) in a total volume of $3cm^3$. The mixtures were incubated at 37° for 15 mins., after which the absorptions at 400nm in 1cm silica cells were determined against a catechol-buffer blank. One unit of laccase activity is defined as that amount of enzyme which will cause the optical density to increase by 1.0 unit after incubation for 15 mins.

Assay of Horseradish Peroxidase

The enzyme was purchased from Sigma Chemical Co., as a salt-free powder, and was assayed for activity according to the method of Devlin.[224] Reaction mixtures contained guaiacol solution (3.3×10^{-4}M, $0.05cm^3$), hydrogen peroxide solution (1.3×10^{-4}M, $0.04cm^3$), peroxidase solution ($0.1g/dm^3$ in pH6.0 buffer; $0.01cm^3$) and phosphate buffer (0.01M, pH6.0, $2.9cm^3$). The absorbance of the mixture was determined after 5 mins. at 470 nm in 1cm silica cells against a blank containing guaiacol, peroxide and buffer. An activity of 1 unit is defined as the amount of enzyme causing an increase in optical density of 1.0 unit in 5 mins.

1. Preparation of Compounds in the 6-Hydroxy-7-Methoxy Series

Benzylisovanillin 334

Isovanillin (125g, 0.52mol), dissolved in meths ($250cm^3$) was added to a stirred mixture of benzyl chloride (83g, 0.66mol), and potassium carbonate (72g) in meths ($200cm^3$). The mixture was refluxed for 10 hr.

with stirring, followed by addition of activated charcoal (5g) and further reflux for 30 mins. The reaction mixture was filtered hot and allowed to cool. The resulting pale yellow crystals were filtered off, washed with petrol (b.p. 40-60°) and recrystallised (ethanol) affording 334 (162g, 81%), m.p. 63-4° (lit 63°)[357] ν_{max}^{KBr}: 1680 (C=O). $\tau(CDCl_3)$: 0.23 (1H, s, CHO); 2.50-3.18 (8H, m, aromatics); 4.90 (2H, s, $PhCH_2O$); 6.13 (3H, s, CH_3O).

3-Benzyloxy-4-methoxy-ω-nitrostyrene 335

Benzylisovanillin (87.5g, 0.36mol), ammonium acetate (29g), and nitromethane (113g, 1.85mol) were refluxed in acetic acid (250cm^3) for 1.5 hr. and allowed to cool overnight. The crystalline product was filtered off, washed thoroughly with ether (500cm^3) and recrystallised (ethanol), giving bright yellow needles of 335 (81g, 79%), m.p. 127.5-8° (lit 125-7°)[358] ν_{max}^{KBr}: 1625 (C=C). $\tau(CDCl_3)$: 1.93-3.15 (10H, m, aromatics and vinylics); 4.85 (2H, s, $PhCH_2O$); 6.08 (3H, s, CH_3O).

3-Benzyloxy-4-methoxy-β-phenylethylamine hydrochloride 336

The above nitrostyrene 335 (60g, 0.21mol) dissolved in dry THF (500cm^3) was added dropwise to a mechanically stirred suspension of lithium aluminium hydride (25g) in THF (500cm^3) under nitrogen. When the addition was complete the mixture was stirred for 1hr., following which portions of aqueous THF (50cm^3 of 20% aq.THF, followed by 50cm^3 of 50% aq.THF) were cautiously added. Addition of 4M sodium hydroxide solution gave a granular precipitate of inorganic salts and a clear organic layer which was decanted off. The inorganic precipitate was washed with THF (3 x 200cm^3) and the washings combined with the

original THF solution. Removal of the organic solvent under reduced pressure gave a brown oil which was dissolved in ether and dried (K_2CO_3). Filtration was followed by the addition of small portions of ethanolic hydrogen chloride, the resulting white fluffy precipitate being collected and washed with ether to give 44.5g (71%) of 336. Recrystallisation of the hydrochloride (ethanol-ether) gave white crystals, m.p. 162-5° (lit 166°).[115] ν_{max}^{KBr}: 2600cm^{-1}, 2400cm^{-1} (salt bands).

3-Hydroxy-4-methoxy-β-phenylethylamine hydrochloride 337

The hydrochloride 336 (22g, 0.075mol) was placed in a 500cm^3 hydrogenation bottle and dissolved in meths (200cm^3) containing 5% palladised charcoal (2g). The system was pressurised with hydrogen and shaken until uptake of gas had ceased. Catalyst was removed by filtration and the solvent removed to give the hydrochloride 337 (14g, 92%), as white crystals, m.p. 203-5° (lit 204-6°)[227] from ethanol-ether. ν_{max}^{KBr}: 3150cm^{-1} (H bonded O-H); 2640, 2560, 2450cm^{-1} (salt bands). $\tau(D_2O)$: 3.11 (3H, s, aromatics); 6.12 (3H, s, $C\underline{H}_3O$); 6.55-7.21 (4H, m, $C\underline{H}_2$-$C\underline{H}_2$).

1.1 1-Alkyl-6-Hydroxy-7-Methoxy-3,4-Dihydroisoquinoline Hydrochlorides

6-Hydroxy-7-methoxy-3,4-dihydroisoquinoline hydrochloride 169

Ethyl formate (60cm^3, 0.74mol) and 3-benzyloxy-4-methoxy-β-phenylethylamine 336 (15g, 0.058mol) were refluxed together for 15 hr. Excess ethyl formate was removed by distillation under reduced pressure, leaving a brown gum which was triturated with ether-petrol (b.p. 40-60°) giving the N-formyl compound 338 (16.3g, 98%). The crude product recrystallised from benzene-petrol (b.p. 60-80°) as a fawn solid, m.p. 98.5-100.5° (lit 101°).[358] ν_{max}^{KBr}: 3380cm^{-1} (N-H); 1660cm^{-1}(C=O). $\tau(CDCl_3)$: 2.00 (1H, s, C$\underline{H}$O); 2.62-3.26 (8H, m, aromatics); 4.23 (1H, broad s, N$\underline{H}$); 4.90 (2H, s, Ph$C\underline{H}_2$O); 6.18 (3H, s, $C\underline{H}_3O$); 6.64, 7.35 (4H, 2t, $C\underline{H}_2$-$C\underline{H}_2$).

The above formamide (10g, 0.036mol) was dissolved in toluene ($100cm^3$) and cooled whilst phosphorus oxychloride ($30cm^3$) was added cautiously. The mixture was refluxed for 1.5hr., cooled, and poured into a large excess of petrol (b.p.60-80°; $600cm^3$) which was decanted off leaving a dark brown gum. After an additional wash with petrol ($400cm^3$), 1M hydrochloric acid ($250cm^3$) was added to the residue and heated at 50° for 30 mins. This procedure was repeated twice more, the combined acid washings then being cooled, basified with 2M sodium hydroxide solution and extracted with ether (3 x $150cm^3$), dried (K_2CO_3) and evaporated. Trituration with petrol (b.p. 60-80°) gave 6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline 292, (5.3g, 57%) as colourless crystals, m.p. 102-3° (lit 105°).[358] $\nu_{max.}^{KBr}$: 1635cm^{-1} (C=N). τ($CDCl_3$): 1.94 (1H, s, C<u>H</u>=N); 2.59-3.26 (7H, m, aromatics); 4.98 (2H, s, PhC<u>H</u>$_2$O); 6.05 (3H, s, C<u>H</u>$_3$O); 6.28, 7.31 (4H, 2t, C<u>H</u>$_2$-C<u>H</u>$_2$).

Compound 292 (0.5g, 1.8 mmol) was refluxed with a 1:1 mixture of ethanol and conc. hydrochloric acid ($40cm^3$) for 30 mins., followed by removal of solvent to afford 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline hydrochloride 169 (0.34g, 85%) as pale yellow crystals (ethanol), m.p. 206-9 (lit 207-8°).[241] ν(nujol): 3200cm^{-1} (O-H); 1640cm^{-1}(C=N). τ(D_2O): 1.20 (1H, s, C<u>H</u>=N); 2.67, 3.20 (2H, 2s, aromatics); 6.07 (3H, s, C<u>H</u>$_3$O); 5.99, 6.90 (4H, 2t, C<u>H</u>$_2$-C<u>H</u>$_2$).

<u>1-Methyl-6-hydroxy-7-methoxy-3,4-dihydroisoquinoline hydrochloride 170</u>

Acetic acid was added dropwise to an ethereal solution of the phenylethylamine 336 (5.25g, 0.02mol) giving a white precipitate which was filtered and air dried. Heating of this salt at 180° on an oil bath for 1.5 hr. followed by dissolving the tan oil in dichloromethane ($50cm^3$), washing with 2M hydrochloric acid (3 x $50cm^3$), H_2O (3 x $50cm^3$), 2M sodium

hydroxide solution (3 x 50cm^3) and drying over $MgSO_4$, afforded after recrystallisation from ethyl acetate 2.6g of acetamide 339 (43%), m.p. 123-5° (lit 128-8.5°).[360] ν_{max}^{KBr}: 3308cm^{-1}(N-H); 1644cm^{-1}(C=O). τ($CDCl_3$): 2.58-3.25 (8H, m, aromatics); 4.55 (1H, broad s, NH); 6.16 (3H, s, CH_3O); 6.65, 7.34 (4H, 2t, CH_2-CH_2); 8.17 (3H, s, CH_3).

The acetamide 339 (2.6g, 8.7mmol) was cyclised with phosphorus oxychloride (10cm^3) as for compound 292. Methanol (5cm^3) was added to the resultant brown gum followed by trituration with ether to give the hydrochloride of 1-methyl-6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline 340 (2.2g, 80%), m.p. 183-4.5° (lit 202-4°),[241] as colourless crystals (ethanol-ether). $\nu_{(nujol)}$: 1650cm^{-1} (C=N). τ($CDCl_3$): 2.46-3.43 (7H, m, aromatics); 4.95 (2H, s, $PhCH_2O$); 6.10 (3H, s, CH_3O); 6.25, 7.40 (4H, 2t, CH_2-CH_2); 7.84 (3H, s, CH_3).

Debenzylation of 340 (2.1g, 6.6mmol) with ethanolic conc. hydrochloric acid (1:1, 15cm^3), as for 169 furnished after crystallisation from ethanol-ether 1.4g (93%) of 170, m.p. 260-2° (lit 261-3°).[241] $\nu_{(nujol)}$: 3420cm^{-1} (O-H); 1650cm^{-1} (C=N). τ(D_2O): 2.73, 3.28 (2H, 2s, aromatics); 6.07 (3H, s, CH_3O); 6.23, 7.00 (4H, 2t, CH_2-CH_2); 7.20 (3H, s, CH_3).

1-Benzyl-6-hydroxy-7-methoxy-3,4-dihydroisoquinoline hydrochloride 171

The above experimental procedure was again adopted to prepare the following compounds. Phenylacetic acid and 336 (10g, 0.039mol) gave the phenylacetamide 341 (8.7g, 63%), m.p. 117-8° (lit 115-7°),[361] as colourless crystals (ethyl acetate). $\nu_{(nujol)}$: 3300cm^{-1} (N-H); 1645cm^{-1} (C=O). τ($CDCl_3$): 2.63-3.36 (13H, m, aromatics); 4.64 (1H, broad s, NH); 4.95 (2H, s, $PhCH_2O$); 6.15 (3H, s, CH_3O); 6.54 (2H, s, $PhCH_2$); 6.68, 7.41 (4H, 2t, CH_2-CH_2). The crude amide 341 (7.5g, 0.02mol) was cyclised to give 1-benzyl-6-benzyloxy-7-methoxy-3,4-dihydroisoquinoline

hydrochloride 342 (5.2g, 66%), m.p.186-91° (lit 204-5°),[361] after crystallisation from ethanol-ether. $\nu_{(nujol)}$: 1645cm^{-1} (C=N). τ($CDCl_3$) as free base: 2.61-3.32 (12H, 4s, aromatics); 4.87 (2H, s, $PhC\underline{H}_2O$); 5.97 (2H, s, $PhC\underline{H}_2$); 6.28 (3H, s, $C\underline{H}_3O$); 6.31, 7.44 (4H, 2t, $C\underline{H}_2$-$C\underline{H}_2$). The hydroxy compound 171 (1.3g, 54%), m.p. 84-6° was obtained from 3.1g (7.8mmol) of 342 using ethanol-conc. hydrochloric acid as the debenzylating medium. ν^{KBr}_{max}: 3390cm^{-1} (O-H); 1640cm^{-1} (C=N). τ(D_2O): 2.50 (6H, s, aromatics); 5.81 (2H, s, $PhC\underline{H}_2$); 6.08 (3H, s, $C\underline{H}_3O$); 6.10, 7.01 (4H, 2t, $C\underline{H}_2$-$C\underline{H}_2$).

1-(3',4',5'-Trimethoxybenzyl)-6-hydroxy-7-methoxy-3,4-dihydroisoquinoline hydrochloride 172

3,4,5-Trimethoxyphenylpyruvic acid 167 was prepared by the following procedure: 3,4,5-trimethoxybenzaldehyde (50g, 0.255mol), hippuric acid (75g, 0.42mol) and sodium acetate (23g) were heated with acetic anhydride (137cm^3) on a steam bath for 30 mins. The dark orange solution was cooled overnight at 4°, the precipitated yellow needles were filtered off, washed with water, meths, and water again to give the desired azlactone 168 (74.7g, 86%), m.p. 163-5° (lit 165-6).[231] ν^{KBr}_{max}: 1780cm^{-1} (C=O). τ($CDCl_3$): 1.70-2.51 (7H, m, aromatics); 2.90 (1H, s, C=C$\underline{H}$); 6.05 (9H, s, $3C\underline{H}_3O$).

Azlactone 168 (30g, 0.088mol) was refluxed for 1hr. in 4M sodium hydroxide solution (150cm^3), after which water (100cm^3) was added and the solution saturated with sulphur dioxide. The precipitated benzoic acid was filtered off giving a pale yellow filtrate which afforded crystals of 3,4,5-trimethoxyphenylpyruvic acid 167 on heating with excess 4M hydrochloric acid. Recrystallisation from aqueous ethanol gave 14.7g (65%) of product as colourless needles, m.p. 166-7° (lit 167-8°).[231]

ν_{max}^{KBr}: 3460cm^{-1} (O-H as enol); 1705cm^{-1} (CO_2H). τ(DMSO): 0.63 (1H, broad s, CO_2H); 2.77 (2H, s, aromatics); 3.50 (1H, s, C<u>H</u>=C-OH); 6.15, 6.25 (9H, 2s, 3C<u>H</u>$_3$O).

Conversion of <u>167</u> to 3,4,5-trimethoxyphenylacetic acid <u>343</u> was achieved by adding dropwise a mixture of hydrogen peroxide (30%, 14cm^3) and water (14cm^3) to a solution of 3,4,5-trimethoxyphenylpyruvic acid <u>167</u> (11.8g, 0.046mol) in 10M sodium hydroxide solution and cooled to 10° in an ice-salt bath; the peroxide solution was added at such a rate that the temperature of the reaction mixture kept below 15°. The solution was stirred overnight at room-temperature before being acidified with conc. hydrochloric acid. The white precipitate obtained was filtered off, washed with water (500cm^3) and air dried, affording 9.4g (90%) of 3,4,5-trimethoxyphenylacetic acid <u>343</u>, m.p. 116-8° (lit 118-20°).[362] ν_{max}^{KBr}: 1700cm^{-1} (CO_2H). τ($CDCl_3$): -1.24 (1H, broad s, exchanges with D_2O, CO_2<u>H</u>); 3.53 (2H, s, aromatics); 6.18 (9H, s, 3C<u>H</u>$_3$O); 6.44 (2H, s, PhC<u>H</u>$_2$).

The title compound was prepared by the same route as the previous dihydroisoquinoline. Heating the salt obtained from 3,4,5-trimethoxyphenylacetic acid <u>343</u> (4.3g, 0.019mol) and amine <u>336</u> (5.0g, 0.019mol) at 180° for 1 hr. gave the <u>substituted phenylacetamide</u> <u>272</u> which recrystallised (ethyl acetate) as buff needles (7.1g, 79%), m.p. 124-6.5°. (Found: C, 69.4; H, 6.7; N, 3.0. $C_{27}H_{31}NO_6$ requires C, 69.7; H, 6.7; N, 3.0%). ν_{max}^{KBr}: 3320cm^{-1} (N-H); 1635cm^{-1} (C=O). τ($CDCl_3$) 2.68-3.69 (10H, m, aromatics); 4.57 (1H, broad s, N<u>H</u>); 4.95 (2H, s, PhC<u>H</u>$_2$O); 6.17, 6.23 (12H, 2s, 4C<u>H</u>$_3$O); 6.63 (2H, s, PhC<u>H</u>$_2$); 6.68, 7.40 (4H, 2t, C<u>H</u>$_2$-C<u>H</u>$_2$). Cyclisation of the crude amide gave 5.3g (72%) of the <u>3,4-dihydroisoquinoline hydrochloride</u> <u>273</u>, m.p. 184-6° (ethanol-

ether). (Found: C, 66.4; H, 6.0; N, 2.8. $C_{27}H_{29}NO_5$.HCl requires C, 67.0; H, 6.2; N, 2.9%). ν_{max}^{KBr}: 2690cm^{-1} (salt band); 1655cm^{-1} (C=N). $\tau(CDCl_3)$: 2.58-3.26 (9H, m, aromatics); 4.83 (2H, s, $PhCH_2O$); 5.42 (2H, s, $PhCH_2$); 6.13, 6.22, 6.28 (12H, 3s, $4CH_3O$); 7.10 (2H, t, CH_2-CH_2-N).

Debenzylation of 273 (1g, 2.1mmol) afforded upon trituration with ethanol-ether a fawn solid of the title compound 172 (0.8g, 98%), m.p. 225-8°. An analytical sample from ethanol-ether melted at 233-6°. (Found: C, 60.9; H, 6.1, N, 3.2. $C_{20}H_{23}NO_5$.HCl requires C, 61.0; H, 6.1; N, 3.5%). ν_{max}^{KBr}: 3450cm^{-1} (O-H); 2750cm^{-1} (salt band); 1650cm^{-1} (C=N). $\tau(D_2O)$: 2.80, 3.18, 3.43 (4H, 3s, aromatics); 6.25 (12H, s, $4CH_3O$); 7.15 (2H, t, CH_2-CH_2-N).

1,2 1-Alkyl-6-Hydroxy-7-Methoxy-1,2,3,4-Tetrahydroisoquinoline-1-Carboxylic Acids

6-Hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 163

A solution of glyoxylic acid monohydrate (100cm^3 of a 1% solution) was added to phenylethylamine 337 (2g, 0.01mol) dissolved in 0.05M sulphuric acid solution (100cm^3). The mixture was heated on a steam bath for 4 hr; the solution was adjusted to pH7 with ammonia solution, reduced to half volume and kept at 4° overnight. The crystalline product was filtered off, dissolved in boiling methanol-conc. hydrochloric acid (100:1) and cooled. Addition of ether gave colourless needles of the 1-hydrogen acid 163 (1.2g, 47%) as the hydrochloride, m.p. 209-12°. (Found: C, 47.8; H, 5.7; N, 4.9. $C_{11}H_{14}ClNO_4.H_2O$ requires C, 47.6; H, 5.8; N, 5.0%). ν_{max}^{KBr}: 3370cm^{-1} (O-H); 2790, 2630, 2530cm^{-1} (salt bands); 1738cm^{-1} (CO_2H). $\tau(D_2O)$: 2.68, 3.09 (2H, 2s, aromatics); 6.02 (3H, s, CH_3O); 6.46 (1H, s, C_1-H); 6.29, 6.94 (4H, 2t, CH_2-CH_2).

1-Methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 164

3-Hydroxy-4-methoxy-β-phenylethylamine hydrochloride (11g, 0.046mol) and pyruvic acid (6.2g, 0.07mol) were dissolved in water ($120cm^3$) and the pH adjusted to 4.5-5.0 with ammonia solution. After standing at 25° for 5 days under N_2 the resulting crystals were collected and recrystallised from methanol-conc.hydrochloric acid-ether to afford the hydrochloride of the title compound 164 (8.2g, 64%) as colourless needles, m.p. 254-6° (d) (lit 254°).[230] ν_{max}^{KBr}: 3340cm^{-1} (O-H); 2790, 2560cm^{-1} (salt bands); 1740cm^{-1} (CO_2H). $\tau(D_2O)$: 2.83, 3.27 (2H, 2s, aromatics); 6.07 (3H, s, $C\underline{H}_3O$); 6.37, 6.97 (4H, 2t, $C\underline{H}_2$-$C\underline{H}_2$); 7.96 (3H, s, $C\underline{H}_3$).

1-Benzyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 165

Phenylpyruvic acid (1.6g, 0.01mol) dissolved in the minimum amount of 1M sodium hydroxide solution and 3-hydroxy-4-methoxy-β-phenylethylamine hydrochloride (2g, 0.01mol) in water ($30cm^3$) were allowed to stand under N_2 for 24 hr., the pH having been adjusted to 6.5-7.0 with 2M hydrochloric acid. White crystalline material was filtered off, and recrystallised (methanol-conc. hydrochloric acid-ether) to give the hydrochloride of 165 (1.7g, 55%) as off-white crystals, m.p. 245-9°(d). (Found: C, 61.4; H, 5.8; N, 3.8. $C_{18}H_{19}NO_4.HCl$ requires C, 61.8; H, 5.7; N, 4.0%). ν_{max}^{KBr}: 3480cm^{-1} (O-H); 3170cm^{-1} ($\overset{+}{N}H_2$); 2730, 2520, 2400cm^{-1} (salt bands); 1710cm^{-1} (CO_2H). $\tau(D_2O)$: 2.93-3.56 (7H, m, aromatics), 6.10 (3H, s, $C\underline{H}_3O$).

1-(3′,4′,5′-Trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 166

The title acid was prepared in an identical manner to the preceeding 1-benzyl analogue from phenylethylamine 337 (6.1g, 0.03mol) dissolved

in water ($30cm^3$) and 3,4,5-trimethoxyphenylpyruvic acid 167 (7.6g, 0.03mol). The crystalline product was collected after 5-6 days, recrystallised from methanol, conc. hydrochloric acid and ether to afford the hydrochloride of title compound 166 (6.7g, 56%) as colourless needles, m.p. 252-4°(d). (Found: C, 56.9; H, 6.1; N, 2.7. $C_{21}H_{25}NO_7.HCl$ requires C, 57.3; H, 5.9; N, 3.2%). ν_{max}^{KBr}: 3320cm^{-1} (O-H); 3160cm^{-1} ($\overset{+}{N}H_2$); 2665, 2530, 2430cm^{-1} (salt bands); 1710cm^{-1} (CO_2H). $\tau(D_2O)$: 2.48, 3.10, 3.39 (4H, 3s, aromatics); 5.93 (2H, s, $PhCH_2$); 6.02, 6.15 (12H, 2s, $4CH_3O$); 6.40, 7.03 (4H, 2t, CH_2-CH_2).

2. Preparation of Compounds in the 6,7-Dimethoxy Series

2.1 1-Alkyl-6,7-Dimethoxy-3,4-Dihydroisoquinoline Hydrochlorides

6,7-Dimethoxy-3,4-dihydroisoquinoline hydrochloride 173

The title compound 173 was prepared in an analogous fashion to compound 170. The salt produced from 3,4-dimethoxy-β-phenylethylamine (homovertrylamine) and formic acid gave the N-formyl derivative 344, m.p. 38-40° (lit 40-2°).[362] $\nu_{(nujol)}$: 3330cm^{-1} (N-H); 1655cm^{-1} (C=O). $\tau(CDCl_3)$: 1.94 (1H, s, CHO); 2.65-3.31 (3H, 3s, aromatics); 4.46 (1H, broad s, NH); 6.10,6.14 (6H, 2s, $2CH_3O$); 6.51, 7.32 (4H, 2t, CH_2-CH_2). Cyclisation of this amide with phosphorus oxychloride afforded 173 as pale yellow crystals, m.p. 110-2°, picrate, m.p. 200-1° (lit 201-3°),[362] from methanol-ether. ν_{max}^{KBr}: 1630cm^{-1} (C=N). $\tau(CDCl_3)$: 1.98 (1H, s, CH=N); 2.86, 3.20 (2H, 2s, aromatics); 6.08 (3H, s, CH_3O); 6.28, 7.24 (4H, 2t, CH_2-CH_2).

1-Methyl-6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride 174

The N-acetyl derivative 345 was prepared via the salt of homoveratrylamine with glacial acetic acid as a fawn coloured solid (ethyl acetate), m.p.

94-6° (lit 94-5°).[362] ν_{max}^{KBr}: 3270cm^{-1}(N-H); 1640cm^{-1}(C=O). τ($CDCl_3$): 3.18-3.21 (3H, 2s, aromatics); 6.12 (6H, s, 2C$\underline{H}_3$O); 6.56, 7.25 (4H, 2t, C$\underline{H}_2$-C$\underline{H}_2$); 8.06 (3H, s, C$\underline{H}_3$). The acetamide 345 was cyclised with phosphorus oxychloride in toluene to give 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride 174 as pale yellow needles, m.p. 195-6° (lit 200°).[363] ν_{max}^{KBr}: 1635cm^{-1} (C=N). τ($CDCl_3$): 2.95, 3.25 (2H, 2s, aromatics); 6.07 (6H, s, 2C$\underline{H}_3$O); 6.33, 7.38 (4H, 2t, C$\underline{H}_2$-C$\underline{H}_2$); 7.62 (3H, s, C$\underline{H}_3$).

1-Benzyl-6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride 175

Heating of the salt from phenylacetic acid and homoveratrylamine (10g, 0.055mol) at 180° for 1.5 hr. produced 16.1g (91%) of the phenylacetamide 346 as a beige solid, m.p. 109-110° (lit 111°)[364] from ethyl acetate. $\nu_{(nujol)}$: 3325cm^{-1} (N-H); 1630cm^{-1} (C=O). τ($CDCl_3$): 2.68-3.34 (8H, m, aromatics); 4.48 (1H, broad s, N$\underline{H}$); 6.12, 6.16 (6H, 2s, 2C$\underline{H}_3$O); 6.46 (2H, s, PhC$\underline{H}_2$); 6.59, 7.32 (4H, 2t, C$\underline{H}_2$-C$\underline{H}_2$). The amide was cyclised by the phosphorus oxychloride-toluene method to afford 68% of title compound 175 as colourless needles (ethanol-ether), m.p. 175-6° (lit 175°).[364] $\nu_{(nujol)}$: 2650cm^{-1} (salt band); 1642cm^{-1} (C=N). τ($CDCl_3$) as free base: 2.76, 3.07, 3.38 (7H, 3s, aromatics); 5.98 (2H, s, PhC$\underline{H}_2$); 6.18, 6.32 (6H, 2s, 2C$\underline{H}_3$O); 6.28, 7.45 (4H, 2t, C$\underline{H}_2$-C$\underline{H}_2$).

2.2 1-Alkyl-6,7-Dimethoxy-1,2,3,4-Tetrahydroisoquinoline-1-Carboxylic Acids

N-Benzoyl-1-cyano-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 182

The hydrochloride 173 (8.3g, 0.037mol) was dissolved in water (60cm^3) which was made basic with 4M sodium hydroxide solution and extracted with dichloromethane (3x25cm^3). The combined organic extracts were dried ($MgSO_4$) and filtered before being mixed with a solution of

potassium cyanide (12.0g dissolved in the minimum amount of water) and stirred magnetically whilst benzoyl chloride (16g) was added dropwise. After stirring for 3hr. the organic layer was washed with 2M sodium hydroxide (2 x 50cm^3), 2M hydrochloric acid (2 x 50cm^3), water (3 x 50cm^3) and dried ($MgSO_4$). Removal of solvent and trituration with methanol gave a solid which was recrystallised from methanol to furnish N-benzoyl-1-cyano-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 182 (6.3g, 54%), m.p. 215-7° (lit 215-6°)[234] as colourless crystals. ν_{max}^{KBr}: 1655cm^{-1} (C=O). $\tau(CDCl_3)$: 2.52, 3.22, 3.35 (7H, 3s, aromatics); 3.77 (1H, broad s, C-1H); 6.14 (6H, s, $2CH_3O$); 6.41, 7.15 (4H, 2t, CH_2-CH_2).

N-Benzoyl-1-cyano-1-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 183

The Reissert compound 182 (2.0g, 6.2mmol) and a dispersion of sodium hydride in oil (0.35g, 0.012mol) were stirred together in dry DMF (20cm^3). Iodomethane (1.4g, 0.01mol) was added dropwise to the magnetically stirred deep red solution of the Reissert anion, stirring being continued for a further hour; the mixture was poured on to crushed ice (100g) and extracted with chloroform (3 x 100cm^3). The combined extracts were washed with 2M hydrochloric acid (2 x 100cm^3), 2M sodium hydroxide (2 x 100cm^3), water (3 x 100cm^3), dried over $MgSO_4$ and evaporated. Trituration with, and recrystallisation from methanol, gave the title compound 183 as colourless needles, (1.4g, 67%), m.p. 204-6° (lit 205-6°).[242] ν_{max}^{KBr}: 2220cm^{-1} (C≡N); 1650cm^{-1} (C=O). $\tau(CDCl_3)$: 2.50, 2.96, 3.38 (7H, 3s, aromatics); 6.06 (6H, s, $2CH_3O$); 6.38, 7.17 (4H, 2t, CH_2-CH_2); 7.85 (3H, s, CH_3).

Attempts to prepare 183 from 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinoline 174 (1g, 5mmol), potassium cyanide (1.5g) and benzoyl

chloride (2.0g) by the normal Reissert method produced a white solid (0.73g) which t.l.c. (benzene-ether, 2:1) showed to contain two components. The major component had an R_f value of 0.30 and the minor an R_f of 0.10. Separation was achieved by chromatography on a silica-gel column (25g) with benzene-ether (2:1) as eluant. The minor component was identified as 183 (0.13g, 8%), m.p. 198-200°, by comparison with an authentic sample. The major fraction gave a buff coloured solid which after recrystallisation (benzene) afforded 0.58g of colourless crystals, m.p. 147-9°. ν_{max}^{KBr}: 3320cm^{-1} (N-H); 1670cm^{-1} (C=O); 1630cm^{-1} (C=O). $\tau(CDCl_3)$ 2.01-2.74 (7H, m, aromatics); 6.08 (6H, s, 2C$\underline{H}_3$O); 6.26, 6.87 (4H, 2t, C$\underline{H}_2$-C$\underline{H}_2$); 7.37 (3H, s, C$\underline{H}_3$). These spectral data suggested that the product was N-benzoyl-β(2-acetyl-4,5-dimethoxyphenyl)-ethylamine 185, and this was confirmed by comparison with an authentic sample prepared according to the method of Forbes et al.[236]

1-Methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 180

The 1-methyl Reissert compound 183 (0.6g, 2 mmol) was heated on an oil bath at 100° with 85% phosphoric acid (7cm^3) for 1hr. under a stream of N_2. The precipitated benzoic acid produced on addition of the reaction mixture to ice-water, was filtered off and the pH of the filtrate carefully adjusted to 7.0 with conc. ammonia solution. A white crystalline precipitate was formed which was filtered off, giving colourless crystals of title compound 180 (0.23g, 51%), m.p. 308-310°(d) (lit 298-300°).[242] Recrystallisation from methanol-conc. hydrochloric acid-ether gave the hydrochloride salt, m.p. 258-60°(d). ν_{max}^{KBr}: 2840, 2780cm^{-1} (salt bands); 1742cm^{-1} (CO_2H). $\tau(D_2O)$: 2.68, 2.96 (2H, 2s, aromatics); 5.94, 6.02 (6H, 2s, 2C$\underline{H}_3$O); 6.20, 6.77 (4H, 2t, C$\underline{H}_2$-C$\underline{H}_2$): 7.84 (3H, s, C$\underline{H}_3$).

N-Benzoyl-1-benzyl-1-cyano-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 184

The 1-benzyl Reissert compound 184 was prepared by the method described for compound 183. Reaction of the isoquinoline 173 (1.0g, 3mmol), sodium hydride (0.5g, 0.01mol) and benzyl chloride (0.7g, 5.5mmol) produced 0.5g (39%) of 184 after recrystallisation from methanol, m.p. 184-7° (lit 185-6°).[234] ν_{max}^{KBr}: 2200cm^{-1} (C≡N); 1640cm^{-1} (C=O). τ($CDCl_3$): 3.07-3.87 (12H, m, aromatics); 5.68 (2H, d, $PhC\underline{H}_2$); 6.16 (6H, s, $2C\underline{H}_3O$).

1-Benzyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 181

The amino-acid hydrochloride 181 was prepared in an analogous fashion to 180 by hydrolysis of the 1-benzyl Reissert 184 (0.75g, 1.8mmol) with 85% phosphoric acid (8cm^3) to afford 0.44g (74%) of title compound 181, m.p. 281-6 (d), which upon recrystallisation (ethanol-conc. hydrochloric acid-ether) gave the hydrochloride salt, m.p. 238-40°(d). ν_{max}^{KBr}: 2785, 2700cm^{-1} (salt bands); 1725cm^{-1} (CO_2H). τ(D_2O): 2.76-3.21 (7H, m, aromatics); 6.04, 6.07 (6H, 2s, $2C\underline{H}_3O$); 6.23 (2H, s, $PhC\underline{H}_2$); 6.26, 6.83 (4H, 2t, $C\underline{H}_2$-$C\underline{H}_2$).

3. Preparation of Compounds in the 6-Methoxy-7-Hydroxy Series

3-Methoxy-4-benzyloxy-β-phenylethylamine 347

A solution of vanillin (125g, 0.82mol) in ethanol (500cm^3) was added to a solution of potassium hydroxide (46g) in ethanol (125cm^3) followed by benzyl chloride (113g, 0.89mol). The mixture was refluxed for 4.5 hr. and cooled at 4° overnight. The resultant crystals were filtered, washed with water (1dm^3), 0.1M sodium hydroxide solution (500cm^3) and petrol (b.p. 40-60°) before being air dried, affording 164g (82%) of benzylvanillin 348 as pale yellow crystals (ethanol), m.p. 61.5-63°

(lit 64-5°).[365] ν_{max}^{KBr}: 1680cm^{-1} (C=O). τ($CDCl_3$): 0.34 (1H, s, C$\underline{H}$O); 2.58-3.12 (8H, m, aromatics); 4.95 (2H, s, PhC$\underline{H}_2$O); 6.12 (3H, s, C$\underline{H}_3$O). 3-Methoxy-4-benzyloxy-ω-nitrostyrene 349 was prepared by refluxing benzylvanillin 348 (100g, 0.40mol), nitromethane (120cm^3), ammonium acetate (40g) and acetic acid (400cm^3) for 1.5hr. The dark orange solution was cooled overnight, yielding 91g (77%) of nitrostyrene 349 as yellow platelets (ethanol), m.p. 121-2.5° (lit 121°).[366] ν_{max}^{KBr}: 1635cm^{-1} (C=C). τ($CDCl_3$): 1.88-3.16 (10H, m, aromatics and vinylics); 4.82 (2H, s, PhC$\underline{H}_2$O); 6.10 (3H, s, C$\underline{H}_3$O).

Reduction of 349 to 3-methoxy-4-benzyloxy-β-phenylethylamine 347 was performed in an identical manner to the previously described reduction of 335, giving a 74% yield of the hydrochloride, m.p. 175-8° (lit 176-8°).[367] ν_{max}^{KBr}: 2500-2750cm^{-1} (salt bands)..

3.1 1-Alkyl-6-Methoxy-7-Hydroxy-3,4-Dihydroisoquinoline Hydrochlorides

6-Methoxy-7-hydroxy-3,4-dihydroisoquinoline hydrochloride 176

N-Formyl-3-methoxy-4-benzyloxy-β-phenylethylamine 350 was prepared in quantitative yield from the phenylethylamine 347 and ethyl formate, using the method described for its isomer 338. Recrystallisation from benzene-petrol (b.p. 80-100°) afforded 350 as a beige solid, m.p. 54.5-7.5° (lit 57-63°).[368] ν_{max}^{KBr}: 3300cm^{-1} (N-H); 1640cm^{-1} (C=O). τ($CDCl_3$): 1.91 (1H, s, C$\underline{H}$O); 2.60-3.28 (8H, m, aromatics); 4.03 (1H, broad s, N$\underline{H}$); 4.91 (2H, s, PhC$\underline{H}_2$O); 6.16 (3H, s, C$\underline{H}_3$O); 6.51, 7.28 (4H, 2t, C$\underline{H}_2$-C$\underline{H}_2$). Cyclisation of the amide (15.7g, 0.055mol) was carried out using phosphorus pentachloride (20g) in chloroform (200cm^3), the mixture being allowed to stand at room temperature for 24 hr. Removal of the solvent and trituration with methanol-ether gave a brown solid which was crystallised immediately from ethanol-ether to yield the 3,4-dihydro-

isoquinoline hydrochloride 189 (11.7g, 70%) as colourless crystals, m.p. 178-80° (lit 191-1.5°).[241] ν_{max}^{KBr}: as free base: 1650cm^{-1} (C=N). τ($CDCl_3$): 1.88 (1H, s, C<u>H</u>=N); 2.54-3.37 (7H, m, aromatics); 4.93 (2H, s, PhC<u>H</u>$_2$O); 6.15 (3H, s, C<u>H</u>$_3$O); 6.32, 7.40 (4H, 2t, C<u>H</u>$_2$-C<u>H</u>$_2$).

6-Methoxy-7-hydroxy-3,4-dihydroisoquinoline hydrochloride 176 was made in 87% yield by refluxing the benzyloxy compound 189 in 1:1 ethanol-conc. hydrochloric acid for 30 mins., to give pale yellow crystals after recrystallisation (ethanol), m.p. 237-242° (lit 239-41°).[241] $\nu_{(nujol)}$: 1650cm^{-1} (C=N). τ(D_2O): 1.25 (1H, s, C<u>H</u>=N); 2.88, 3.01 (2H, 2s, aromatics); 5.96 (3H, s, C<u>H</u>$_3$O); 5.98, 6.83 (4H, 2t, C<u>H</u>$_2$-C<u>H</u>$_2$).

<u>1-Methyl-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline hydrochloride</u> 177

The N-acetyl compound 351 was prepared by heating the appropriate phenylethylamine acetate to give a fawn coloured solid (31%), m.p. 110-3° (lit 116-[illegible]°).[369] ν_{max}^{KBr}: 3310cm^{-1} (N-H); 1645cm^{-1} (C=O). τ($CDCl_3$): 2.58-3.27 (8H, m, aromatics); 4.35 (1H, broad s, N<u>H</u>); 4.90 (2H, s, PhC<u>H</u>$_2$O); 6.15 (3H, s, C<u>H</u>$_3$O); 6.61, 7.30 (4H, t, C<u>H</u>$_2$-C<u>H</u>$_2$); 8.13 (3H, s, C<u>H</u>$_3$). Cyclisation of the crude acetamide with phosphorus oxychloride gave 1-methyl-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline 190 in 40% yield, as lemon coloured needles, m.p. 88-89° (lit 87-88°),[370] from petrol (b.p. 60-80°). ν_{max}^{KBr}: 1635cm^{-1} (C=N). τ($CDCl_3$): 2.45-3.27 (7H, m, aromatics); 4.85 (2H, s, PhC<u>H</u>$_2$O); 6.09 (3H, s, C<u>H</u>$_3$O); 6.37, 7.43 (4H, 2t, C<u>H</u>$_2$-C<u>H</u>$_2$); 7.77 (3H, s, C<u>H</u>$_3$). Debenzylation with ethanol-conc. hydrochloric acid yielded 93% of hydrochloride 177, m.p. 220-2° (lit 224-5°)[370] as pale yellow needles from ethanol-ether. ν_{max}^{KBr}: 2730, 2660, 2580cm^{-1} (salt bands); 1645cm^{-1} (C=N). τ(D_2O): 2.77, 3.38 (2H, 2s, aromatics); 6.10 (3H, s, C<u>H</u>$_3$O); 6.34, 7.10 (4H, 2t, C<u>H</u>$_2$-C<u>H</u>$_2$); 7.14 (3H, s, C<u>H</u>$_3$).

1-Benzyl-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline hydrochloride 178

In a similar fashion was prepared the appropriately substituted phenylacetamide 352 as a fawn solid (ethyl acetate), m.p. 97-8° (lit 106-7°).[371] $\nu_{(nujol)}$: 3310cm^{-1} (N-H); 1645cm^{-1} (C=O). $\tau(CDCl_3)$: 2.49-3.61 (13H, m, aromatics); 4.36 (1H, broad s, NH); 4.92 (2H, s, PhCH$_2$O); 6.22 (3H, s, CH$_3$O); 6.53 (2H, s, PhCH$_2$); 6.66, 7.37 (4H, 2t, CH$_2$-CH$_2$). The 7-benzyloxy hydrochloride 353 was prepared in 70% yield by cyclisation using phosphorus oxychloride of amide 352; m.p. 204-6° (lit 210-11°).[371] $\nu_{(nujol)}$: 1642cm^{-1} (C=N). $\tau(CDCl_3)$ as free base: 2.64-3.34 (12H, m, aromatics); 5.02 (2H, s, PhCH$_2$O): 6.09 (2H, s, PhCH$_2$); 6.15 (3H, s, CH$_3$O); 6.28, 7.42 (4H, 2t, CH$_2$-CH$_2$). Refluxing the dihydroisoquinoline 353 with ethanol-conc hydrochloric acid gave the title compound 178 (69%) as a fawn coloured solid (ethanol-ether); m.p. 112-5° (lit 204-5°).[371] ν^{KBr}_{max}: 2850, 2740cm^{-1} (salt bands); 1640cm^{-1} (C=N). $\tau(D_2O)$: 2.49-3.04 (7H, 2s, aromatics) 5.96 (3H, s, CH$_3$O); 5.98 (2H, s, PhCH$_2$); 6.00, 6.90 (4H, 2t, CH$_2$-CH$_2$).

3.2 1-Alkyl-6-Methoxy-7-Hydroxy-1,2,3,4-Tetrahydroisoquinoline-1-Carboxylic Acids

N-Benzoyl-1-cyano-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 186

The Reissert compound 186 was synthesised by the method used for 182. Reaction of potassium cyanide and benzoyl chloride with dihydroisoquinoline 189 (4.2g, 0.016mol) gave 3.6g of a pale yellow solid, m.p. 157-60°, which after two recrystallisations from benzene was shown by t.l.c. (benzene-ether, 2:1) to contain two spots, a major spot of R_f 0.52 and a minor contaminant having an R_f of 0.16. Column chromatography (silica) using benzene-ether (2:1) as eluant gave good separation of these two components. The fast running material was shown to be the title compound 186 (2.9g, 46%), m.p. 182-3° (lit 178-80°)[1] crystallising

as colourless needles from methanol. ν_{max}^{KBr}: 2220cm^{-1} (C≡N); 1645cm^{-1} (C=O). $\tau(CDCl_3)$: 3.00-3.70 (12H, m, aromatics); 4.11 (1H, broad s, C-1H); 5.05 (2H, s, $PhCH_2O$); 6.18 (3H, s, CH_3O); 6.59, 7.15 (4H, 2t, CH_2-CH_2). The minor product was recrystallised from ethyl acetate to furnish yellow crystals of N-benzoyl-2-formyl-4-benzyloxy-5-methoxy-β-phenylethylamine 191 (0.5g, 8%), m.p. 165-6.5°. (Found: C, 72.8; H, 6.1; N, 3.4. $C_{24}H_{23}NO_4$ requires C, 74.1; H, 5.9; N, 3.6%). ν_{max}^{KBr}: 3320cm^{-1} (N-H); 1695cm^{-1} (C=O); 1640cm^{-1} (C=O). $\tau(CDCl_3)$: -0.15 (1H, s, CHO); 2.27-3.16 (12H, m, aromatics); 4.76 (2H, s, $PhCH_2O$); 6.01 (3H, s, CH_3O); 6.25, 6.62 (4H, 2t, CH_2-CH_2).

N-Benzoyl-1-cyano-1-methyl-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 187

Reissert compound 186 (1.7g, 4.3mmol) was suspended in dry DMF (8cm^3). The suspension was stirred magnetically during the addition of sodium hydride (0.26g of an 80% dispersion) over 15 mins producing a deep red coloured solution, typical of Reissert anions. The red colouration disappeared on adding a solution of iodomethane (1g, 7mmol) in DMF (2cm^3), stirring being continued for 4 hr. The DMF solution was poured on to crushed ice (50g) followed by extraction with chloroform (3 x 50cm^3), which was washed with 2M hydrochloric acid (2 x 50cm^3), 2M sodium hydroxide (2 x 50cm^3), water (3 x 50cm^3) and dried ($MgSO_4$) to give on removal of solvent the title compound 187 (1.4g, 80%) as colourless needles (methanol), m.p. 179.5-181°. (Found: C, 75.5; H, 5.7; N, 6.9. $C_{26}H_{24}N_2O_3$ requires C, 75.7; H, 5.8; N, 6.8%). ν_{max}^{KBr}: 2220cm^{-1} (C≡N); 1665cm^{-1} (C=O). $\tau(CDCl_3)$: 2.02-3.08 (12H, m, aromatics); 4.85 (2H, s, $PhCH_2O$); 6.06 (3H, s, CH_3O); 6.25, 6.88 (4H, 2t, CH_2-CH_2); 7.53 (3H, s, CH_3).

The reaction of 1-methyl-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline

190 (3g, 0.011mol) in dichloromethane (50cm^3) with potassium cyanide (3.5g) dissolved in the minimum volume of water and benzoyl chloride (4.8g, 0.034mol) by the method previously described gave colourless crystals of N-benzoyl-2-acetyl-4-benzyloxy-5-methoxy-β-phenylethylamine 192 (2.75g, 64%), m.p. 157-9° (ethyl acetate), as the sole product. (Found: C, 74.3; H, 6.4; N, 3.4. $C_{25}H_{25}NO_4$ requires C, 74.4; H, 6.2; N, 3.5%). ν_{max}^{KBr}: 3325cm^{-1} (N-H); 1675cm^{-1} (C=O); 1635cm^{-1} (C=O). τ($CDCl_3$): 2.06-3.12 (12H, m, aromatics); 4.83 (2H, s, $PhCH_2O$); 6.09 (3H, s, CH_3O); 6.29, 6.91 (4H, 2t, CH_2-CH_2); 7.53 (3H, s, CH_3).

N-Benzoyl-1-benzyl-1-cyano-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 188

The 1-benzyl analogue of 187 was prepared from 186 by the previously described experimental procedure, affording 67% of N-benzoyl-1-benzyl-1-cyano-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 188, m.p. 178.5-180.5° as a colourless solid (methanol). (Found: C, 79.2; H, 5.9; N, 5.7. $C_{32}H_{28}N_2O_3$ requires C, 78.7; H, 5.7; N, 5.7%). $\nu_{(nujol)}$: 2220cm^{-1} (C≡N); 1640cm^{-1} (C=O). τ($CDCl_3$): 3.03-3.87 (17H, m, aromatics); 4.97 (2H, s, $PhCH_2O$); 5.66 (2H, d, $PhCH_2$); 6.15 (3H, s, CH_3O).

Attempts to prepare 1-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid by heating acid 180 (1.2g, 4.9mmol) with 47% hydrobromic acid[241] (12cm^3) for 5 hr. on a steam bath afforded upon removal of solvent and trituration of the residue with ethanol, 1.18g (82%) of a colourless product, m.p. 234-6°(d). ν_{max}^{KBr}: 3575cm^{-1} (O-H); 2860, 2780cm^{-1} (salt bands); 1730cm^{-1} (CO_2H). τ(D_2O): 2.69, 3.06 (2H, 2s, aromatics); 6.30, 6.93 (4H, 2t, CH_2-CH_2); 7.96 (3H, s, CH_3). This product was identified, by comparison with an authentic sample, as 1-methyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrobromide 193.

Reactions for Spectroscopic Assay

(i) Fungal Laccase

The reaction mixture consisted of the isoquinoline-1-carboxylic acid (0.05-1.0cm^3 of an approximately 0.0005M solution), laccase solution (0.1cm^3) and phosphate buffer (0.1M, pH6.0) to a final volume of 3.0cm^3. The increase in absorbance at the relevant wavelength was measured against a blank containing the acid and buffer, but no enzyme.

(ii) Horseradish Peroxidase

Reaction mixtures contained the isoquinoline-1-carboxylic acid (0.05-1.0cm^3 of an approximately 0.0005M solution), hydrogen peroxide solution (0.00087M, 1.0cm^3), peroxidase solution (0.1g/dm^3 in pH6.0 phosphate buffer, 1.0cm^3) and phosphate buffer (0.1M, pH6.0) to a final volume of 3.0cm^3. The increase in absorbance was measured at the appropriate wavelength against an acid, peroxide, buffer blank.

WORK DESCRIBED IN CHAPTER THREE

2-Bromo-3,4,5-trimethoxyphenylacetic Acid 247

Bromine (5.6g) was added dropwise over 30 mins to a stirred solution of 3,4,5-trimethoxyphenylacetic acid (8g, 0.026mol) in acetic acid ($20cm^3$) cooled to 10-15°. Additional stirring for 2 hr., followed by pouring on to crushed ice (50g) afforded a white precipitate which was filtered off, washed with water and recrystallised from chloroform to give 9.8g (91%) of 2-bromo-3,4,5-trimethoxyphenylacetic acid 247, m.p. 150-2°, as colourless crystals. (Found: C, 42.9; H, 4.2; Br, 26.9. $C_{11}H_{13}BrO_5$ requires C, 43.3; H, 4.3; Br, 26.2%). ν_{max}^{KBr}: $1710cm^{-1}$ (CO_2H). $\tau(CDCl_3)$: -0.4 (1H, broad s, exchanges with D_2O, $CO_2\underline{H}$); 3.41 (1H, s, aromatic); 6.17, 6.20 (9H, 2s, $3C\underline{H}_3O$); 6.25 (2H, s, $PhC\underline{H}_2$).

The attempted preparation of title compound 247 using bromine (5g) and 3,4,5-trimethoxyphenylacetic acid (7g, 0.031mol) in undistilled chloroform ($100cm^3$), gave after stirring for 2 hr. and removal of the solvent, a yellow oil (5.7g) which was shown by t.l.c. (chloroform-methanol, 50:1) to contain two spots of approximately equal proportions having R_f's of 0.61 and 0.36. Separation on a silica-gel column (150g) with chloroform as eluant gave pure material (3.2g) of higher R_f which after recrystallisation from petrol (bp 40-60°) was identified as ethyl 2-bromo-3,4,5-trimethoxyphenylacetate 248, m.p. 58.5-60°. (Found: C, 47.1; H, 5.1; Br, 24.0. $C_{13}H_{17}BrO_5$ requires C, 46.9; H, 5.1; Br, 24.0%). ν_{max}^{KBr}: $1735cm^{-1}$ (CO_2Et). $\tau(CDCl_3)$: 3.37 (1H, s, aromatic); 5.86 (2H, q, $CH_3C\underline{H}_2$); 6.15, 6.17 (9H, 2s, $3C\underline{H}_3O$); 6.28 (2H, s, $PhC\underline{H}_2$); 8.74 (3H, t, $C\underline{H}_3$-CH_2). The slower running material was identified as the brominated acid 247, m.p. 150-1°.

N-(3,4-Dimethoxy-β-phenylethyl)-2'-bromo-3',4',5'-trimethoxyphenylacetamide 249

The bromo acid 247 (12.1g, 0.04mol) was dissolved in ether (500cm^3) with homoveratrylamine being added dropwise until precipitation of the white salt was complete. The salt was filtered off, air dried, heated at 180° for 1 hr. to produce a tan oil, which was dissolved in dichloromethane (150cm^3), washed with water (3 x 100cm^3) and dried ($MgSO_4$). Recrystallisation from benzene-petrol (b.p. 80-100°) gave colourless crystals of 249, (16.7g, 90%), m.p. 107-110°. An analytical sample had m.p. 110.5-112°. (Found: C, 53.6; H, 5.6; N, 3.4; Br, 17.0. $C_{21}H_{26}BrNO_6$ requires C, 53.8; H, 5.6; N, 3.0; Br, 17.0%). ν_{max}^{KBr}: 3260cm^{-1} (N-H); 1645cm^{-1} (C=O). τ($CDCl_3$): 3.20-3.52 (4H, m, aromatics); 4.44 (1H, broad s, NH); 6.14-6.20 (15H, 4s, 5CH_3O); 6.38 (2H, s, $PhCH_2$); 6.58-7.31 (4H, 2t, CH_2-CH_2).

In an alternative route to the above substituted phenylacetamide, solutions of homoveratrylamine (4.8g, 0.026mol) and compound 247 (8.0g, 0.026mol) in dichloromethane (40cm^3) were added slowly to a stirred solution of dicyclohexylcarbodiimide (9.0g, 0.04 mol) in dichloromethane (40cm^3) at 0°. The mixture was stirred for an additional 12 hr. at room temperature before acetic acid (2cm^3) was added to destroy excess diimide. Removal of the precipitated N,N'-dicyclohexylurea, followed by washing of the organic phase with 2M hydrochloric acid (3 x 50cm^3), dilute ammonia solution (3 x 50cm^3), water (3 x 50cm^3) and drying ($MgSO_4$) afforded upon removal of the dichloromethane a cream solid (7.9g), m.p. 158-60°, when triturated with ether. The solid contained two components (t.l.c., chloroform, silica-gel), which were separated by preparative t.l.c. using chloroform. Both fractions crystallised from aqueous ethanol as colourless crystals. The slower running, major

component was identified as N-(2-bromo-3,4,5-trimethoxyphenylacetyl)-N,N'-dicyclohexylurea 250, m.p. 161-2°. (Found: Br, 16.3. $C_{24}H_{35}BrN_2O_5$ requires Br, 15.7%). (Found m/e: 512.1637, 510.1675 (calc for $C_{24}H_{35}BrN_2O_5$, 512.1712, 510.1732)). ν_{max}^{KBr}: 3300cm^{-1} (N-H); 1705cm^{-1} (C=O); 1660cm^{-1} (C=O). τ($CDCl_3$): 3.38 (1H, s, aromatic); 6.12, 6.15 (9H, 2s, $3CH_3O$); 6.18 (2H, s, $PhCH_2$); 7.87-9.03 (22H, m, cyclohexyl's). A faster running minor constituent was identified as the dibromo analogue 251 of the above compound, m.p. 189-90°. (Found: Br, 27.7. $C_{24}H_{34}Br_2N_2O_5$ requires Br, 27.1%). ν_{max}^{KBr}: 3280cm^{-1} (N-H); 1700cm^{-1} (C=O); 1660cm^{-1} (C=O). τ($CDCl_3$): 5.78 (2H, s, $PhCH_2$); 6.12, 6.16 (9H, 2s, $3CH_3O$); 7.86-9.03 (22H, m, cyclohexyl's).

1-(2'-Bromo-3',4',5'-trimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride 252

The title dihydroisoquinoline 252 was synthesised from the brominated amide 249 (0.95g, 2mmol) dissolved in dry chloroform (10cm^3) with phosphorus pentachloride (1.3g) being added to the cooled solution. The reaction mixture was left overnight, and the solvent was removed under reduced pressure to afford a brown gum, which on trituration with ether gave a fawn solid. Immediate recrystallisation from ethanol-ether afforded 1-(2'-bromo-3',4',5'-trimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride 252 (0.60g, 61%) as cream coloured crystals, m.p. 207-9°. An analytical sample of 252 as the free base had a melting point of 107-9°, (petrol (b.p. 60-80°)). (Found: C, 56.1; H, 5.2; N, 3.1; Br, 17.4. $C_{21}H_{24}BrNO_5$ requires C, 56.0; H, 5.3; N, 3.1; Br, 17.8%). ν_{max}^{KBr}: 1625cm^{-1} (C=N). τ($CDCl_3$): 3.05-3.32 (3H, 3s, aromatics); 5.85 (2H, s, $PhCH_2$); 6.10-6.26 (17H, m, $5CH_3O$ and CH_2-CH_2-N); 7.36 (2H, t, CH_2-CH_2-N).

N-Trifluoroacetyl-1-(2'-bromo-3',4',5'-trimethoxybenzylidene)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 253

The dihydroisoquinoline hydrochloride 252 (7.2g, 0.015mol) was dissolved in dry chloroform ($70cm^3$). Triethylamine (3g) was added and the solution cooled in an ice-water bath for 15 mins. with stirring. Trifluoroacetic anhydride (24g, 0.114mol) was added cautiously over 15 mins. followed by additional stirring for 4 hr. under nitrogen at room temperature. The reaction mixture was washed with water ($3 \times 30cm^3$), 2M hydrochloric acid ($3 \times 25cm^3$) and dried over $MgSO_4$. Removal of the chloroform afforded a pale yellow solid, m.p. 128.5-140°, which after recrystallisation from ethanol gave colourless crystals of the title compound 253 (3.8g, 47%), m.p. 154-5°. (Found: C, 50.2; H, 4.3; N, 2.4; Br, 14.4. $C_{23}H_{23}BrF_3NO_6$ requires C, 50.5; H, 4.2; N, 2.6; Br, 14.6%). Found m/e = 547.0657, 545.0688 (calculated for $C_{23}H_{23}BrF_3NO_6$, 547.0643, 545.0662). ν_{max}^{KBr}: $1695cm^{-1}$ ($COCF_3$); $1645cm^{-1}$ (C=C). $\tau(CDCl_3)$: 2.78-3.39 (4H, m, aromatics and vinylic); 6.04, 6.10, 6.20 (15H, 3s, $5CH_3O$); 6.98 (2H, t, CH_2-CH_2-N). ^{19}F n.m.r. showed two singlets at 70.19 and 70.87 ppm respectively to high field of the fluorinated chloroform standard.

Using pyridine (either in the cold or under reflux) as the solvent, instead of chloroform in the above reaction gave after work-up by pouring into chloroform and the same procedure, a two spot system (t.l.c., chloroform, silica-gel); the major substance had an R_f = 0.38 and the minor one an R_f = 0.17. Preparative t.l.c. (chloroform-silica-gel) showed the top spot to be the desired product, i.e. 253, m.p. 151-3°. The yellow slower running spot was, on the basis of the spectroscopic evidence identified as 1-(2'-bromo-3',4',5'-trimethoxybenzoyl)-6,7-dimethoxy-3,4-dihydroisoquinoline 254, m.p. 137.5-139°, from ether. (Found: C, 52.8; H, 4.6; N, 2.7. $C_{21}H_{22}BrNO_6$ requires C, 54.3; H, 4.7; N, 3.0%).

Found m/e (Chemical Ionisation) = 464. Major fragments from high resolution mass spectrometry were 384.1457 (100%); 272.9755 (calculated for $C_{21}H_{22}NO_6^{\oplus}$ 384.1448 and for $C_{10}H_{10}BrO_4^{\oplus}$ 272.9822). ν_{max}^{KBr}: 1660cm^{-1} (C=O). τ($CDCl_3$): 2.80, 2.97, 3.28 (3H, 3s, aromatics); 6.07-6.29 (17H, m, $5CH_3O$ and CH_2-CH_2-N); 7.29 (2H, t, CH_2-CH_2-N). ^{19}F n.m.r. gave no absorptions over the usual range.

N-Ethoxycarbonyl-1-(2'-bromo-3',4',5'-trimethoxybenzylidene)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 255

To a stirred solution of the dihydroisoquinoline hydrochloride 252 (2.0g, 4mmol) in chloroform (30cm^3) and 10% sodium carbonate solution (30cm^3) was added ethyl chloroformate (1.81g, 0.017mol) dissolved in chloroform (8cm^3) over 20 mins. Stirring was continued for a further 3 hr. at room temperature, after which the organic layer was washed with water (3 x 25cm^3) 2M hydrochloric acid (3 x 25cm^3), and dried ($MgSO_4$). Removal of the solvent and trituration of the resulting oil with ice cold ethanol gave 0.75g (35%) of the title compound 255, m.p. 97-9°, as colourless crystals (ethanol). (Found: C, 55.2; H, 5.4; N, 2.7; Br, 15.3). $C_{24}H_{28}BrNO_7$ requires C, 55.0; H, 5.4; N, 2.6; Br, 15.3%). ν_{max}^{KBr}: 1690cm^{-1} (C=O). τ($CDCl_3$): 2.68, 3.02, 3.08, 3.35 (4H, 4s, aromatics and vinylic); 6.03-6.38 (19H, m, $5CH_3O$, CH_2=CH_3 and CH_2-CH_2-N); 7.10 (2H, t, CH_2-CH_2-N); 9.17 (3H, t, CH_2-CH_3).

Photolysis of the trifluoroacetamide 253

To a solution of 253 (1.0g, 1.8mmol) dissolved in dry benzene (950cm^3) was added a solution of potassium tertiary butoxide (0.8g) in tert-butanol (50cm^3); the colour of the solution immediately turned yellow. T.l.c. (chloroform-petrol, alumina) showed a complete absence of starting material. The benzene phase was washed with water (3 x 500cm^3), dried

($MgSO_4$) and evaporated to dryness, affording 0.8g of a yellow gum. Trituration with ether gave pale yellow crystals, m.p. 134-8° which were identical both by t.l.c. (chloroform-petrol (b.p. 60-80°), alumina) and by infrared spectroscopy with 1-(2'-bromo-3',4',5'-trimethoxybenzoyl)-6,7-dimethoxy-3,4-dihydroisoquinoline 254. By adding potassium tert-butoxide (0.15g) to a solution of the N-trifluoroacetyl compound 253 (0.2g) in benzene ($75cm^3$) and tert-butanol ($15cm^3$), it was demonstrated that complete conversion to the keto-imine 254 occurred almost immediately.

Photolysis of 253 (0.8g, 1.5mmol) in dry methanol ($550cm^3$) containing calcium carbonate (1.0g) was carried out for 5.5 hr. after which time t.l.c. showed the absence of any starting material. The calcium carbonate was filtered off and methanol removed by distillation under reduced pressure to give 0.50g of an intractable brown gum.

Irradiation of 253 (0.60g, 0.0011mol) in benzene ($1dm^3$) containing calcium carbonate (0.5g) for 12 hr. followed by the previously described work-up procedure gave 0.55g of a dark brown solid from which 0.15g of crystalline material, m.p. 130-5°, identical to compound 254 was obtained on recrystallisation from ethyl acetate. Evaporation of the solvent gave 0.40g of brown solid which t.l.c. (ethyl acetate-petrol (b.p. 60-80°), 3:1) showed to be a multi-component mixture.

Photolysis of carbonate 255

The N-ethoxycarbonyl compound 255 (0.8g, 1.5mmol) dissolved in benzene ($900cm^3$) containing potassium tertiary butoxide (0.75g) and tert-butanol ($100cm^3$) was irradiated under nitrogen for 10 days. The benzene was washed with water (3 x $500cm^3$) and dried ($MgSO_4$). Removal of solvent

gave a brown gum (0.70g) which was shown by t.l.c. to contain two components of R_f 0.53 and R_f 0.42 (ethyl acetate-petrol (b.p. 60-80°), 2:1, silica-gel). Separation was achieved by column chromatography on alumina (21g) with petrol (b.p. 60-80°)—ethyl acetate (2:1) as eluant, giving 0.32g of starting material after trituration with ethanol, m.p. 95-7°, and 0.30g of a yellow oil which could not be made to crystallise. T.l.c. showed this oil to contain some base line material, removal of which was achieved by preparative t.l.c. furnishing 0.13g of a pale yellow oil, which would not solidify. $\nu_{(nujol)}$: 1685cm^{-1} (C=O), UV (ethanol): 265nm; 296nm.

N-(3-Methoxy-4-benzyloxy-β-phenylethyl)-3',4',5'-trimethoxyphenyl-acetamide 257

An ethereal solution of 3,4,5-trimethoxyphenylacetic acid was added dropwise to an ethereal solution of amine 347 (6.1g, 0.024mol) until precipitation of the salt was complete. Heating of the salt at 180° for 1 hr. gave a tan coloured oil which was dissolved in dichloromethane (100cm^3), washed with water (3 x 50cm^3) and dried over $MgSO_4$, to afford on removal of the dichloromethane, 9.7g (88%) of amide 257, m.p. 91-2° (lit 93-4°),[292] as buff crystals (ethyl acetate). ν^{KBr}_{max}: 3300cm^{-1} (N-H); 1645cm^{-1} (C=O). $\tau(CDCl_3)$: 2.69-3.70 (10H, m, aromatics); 4.60 (1H, broad s, exchanges with D_2O, NH); 4.95 (2H, s, $PhCH_2O$); 6.22, 6.27 (12H, 2s, $4CH_3O$); 6.59 (2H, s, $PhCH_2$); 6.65, 7.38 (4H, 2t, CH_2-CH_2).

1-(3',4',5'-Trimethoxybenzyl)-6-methoxy-7-benzyloxy-3,4-dihydroisoquinoline hydrochloride 258

Title compound 258 (7.3g, 96%) was obtained from the phosphorus oxychloride cyclisation of amide 257 (7.3g, 0.016mol) in dichloromethane. Recrystallisation from methanol gave pale yellow crystals, m.p. 198-9° (lit 203-4°).[292] ν^{KBr}_{max}: 1660cm^{-1} (C=N). $\tau(CDCl_3)$ as free base: 2.61-3.48 (9H, m, aromatics); 4.97 (2H, s, $PhCH_2O$); 6.04-6.35 (16H, m, $4CH_3O$, $PhCH_2$, and

CH_2-CH_2-N); 7.23 (2H, t, CH_2-CH_2-N).

1-(3',4',5'-Trimethoxybenzyl)-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline hydrochloride 260

Debenzylation of 258 (3.1g, 6mmol) in 1:1 ethanol-conc. hydrochloric acid ($50cm^3$) gave after removal of solvent under reduced pressure and trituration with ether, 2.4g (95%) of phenolic dihydroisoquinoline 260, m.p. 85-93°, as colourless needles (ethanol). (Found: C, 60.3; H, 6.2; N, 3.4. $C_{20}H_{24}ClNO_5 \cdot \frac{1}{4}H_2O$ requires C, 60.3; H, 6.2; N, 3.5%). ν_{max}^{KBr}: 2840, 2800, 2740cm^{-1} (salt bands); 1640cm^{-1} (C=N). $\tau(D_2O)$: 2.53-3.21 (4H, 3s, aromatics); 5.98, 6.04, 6.17 (14H, m, $4CH_3O$ and $PhCH_2$); 6.86 (2H, t, CH_2-CH_2-N).

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzylidene)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 259

To a solution of the dihydroisoquinoline hydrochloride 258 (2.8g, 5.8mmol) in dry chloroform ($30cm^3$) stirred and cooled to 10°, was cautiously added triethylamine ($3cm^3$) and trifluoroacetic anhydride ($5cm^3$); the solution was stirred for an additional 4 hr, at room temperature, washed with water (3 x $15cm^3$) and 2M hydrochloric acid (3 x $15cm^3$) to give after removal of solvent, a brown oil which on trituration with ice cold ethanol yielded title compound 259 (1.4g, 45%) as colourless crystals (ethanol), m.p. 178-9°. (Found: C, 63.5; H, 5.3; N, 2.5. $C_{29}H_{28}F_3NO_6$ requires C, 64.0; H, 5.2; N, 2.6%). ν_{max}^{KBr}: 1700cm^{-1} ($COCF_3$). $\tau(CDCl_3)$: 2.55-3.45 (10H, m, aromatics and vinylic); 4.81 (2H, s, $PhCH_2O$); 6.15 (12H, s, $4CH_3O$); 6.58, 6.98 (4H, 2t, CH_2-CH_2).

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzylidene)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline 256

To a solution of 259 (2.0g, 3.7mmol) in ethyl acetate ($250cm^3$) was added 5% palladised charcoal (1.5g). The reaction mixture was shaken in a

hydrogenator until uptake of hydrogen had ceased. Removal of the catalyst by filtration followed by evaporation of the solvent gave a colourless glass which was triturated with ice-cold ethanol, to afford colourless crystals of debenzylated material 256 (1.3g, 78%), m.p. 161-3°, (ethyl acetate-petrol (b.p. 60-80°)). (Found: C, 57.9; H, 4.9; N, 2.9. $C_{22}H_{22}F_3NO_6$ requires C, 58.2; H, 4.9; N, 3.1%). ν_{max}^{KBr}: 3390cm^{-1} (O-H); 1705cm^{-1} ($COCF_3$); 1635cm^{-1} (C=C). τ($CDCl_3$): 2.70-3.50 (5H, m, aromatics and vinylic); 4.23 (1H, s, exchanges with D_2O, OH); 6.15 (12H, s, $4CH_3O$); 6.65, 7.00 (4H, 2t, CH_2-CH_2).

An alternative route to 256 involved the reaction of 1-(3',4',5'-trimethoxybenzyl)-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline hydrochloride 260 (1.0g, 2.5mmol) with trifluoroacetic anhydride (6.0g, 0.028mol)in chloroform (25cm^3) and triethylamine (1cm^3). The mixture was left overnight, washed with water (2 x 10cm^3), 2M hydrochloric acid (3 x 10cm^3) and dried ($MgSO_4$). Removal of the solvent, and trituration of the resulting oil with ether, followed by crystallisation from chloroform-petrol, gave 0.6g of a tan solid, m.p. 196-9°. ν_{max}^{KBr}: 1800cm^{-1} (O-$COCF_3$); 1685cm^{-1} (N-$COCF_3$); 1640cm^{-1} (C=C). T.l.c. (ethyl acetate-petrol (b.p. 60-80°) 3:1, silica-gel) showed the product to contain two components (R_f 0.59 and 0.49). The slower running material had the same R_f as genuine 7-hydroxy material 256. The major, faster running component was considered to be the 7-trifluoroacetate 261 on the basis of the infrared evidence. Attempts to obtain pure 261 by preparative t.l.c. resulted in its decomposition to give pure 256, m.p. 160.5-2°. Conversion of the crude trifluoroacetate (1.5g) into the desired phenolic form was carried out by heating with 10% aqueous sodium carbonate (150cm^3) on a steam bath for 15 mins. Acidification of the

reaction mixture followed by extraction with chloroform (3 x 50cm^3) gave after drying ($MgSO_4$) and removal of solvent, 0.80g of pale yellow material, m.p. 152-7° shown by t.l.c.(ethyl acetate-petrol, 3:1) to contain an approximately 1:1 mixture of 256 and a new slower running compound (R_f 0.25). Separation by thick layer chromatography enabled pure unknown material to be obtained and identified as 1-(3',4',5'-trimethoxybenzoyl)-6-methoxy-7-hydroxy-3,4-dihydroisoquinoline 262, m.p. 176-8°(ethyl acetate-petrol). (Found: C, 63.9; H, 5.9; N, 3.6. $C_{20}H_{21}NO_6$ requires C, 64.7; H, 5.7; N, 3.8%). Found m/e = 371 (calc for $C_{20}H_{21}NO_6$, 371). ν_{max}^{KBr}: 2742, 2660, 2617cm^{-1} (salt bands); 1665cm^{-1} (C=O); 1623cm^{-1} (C=N). τ($CDCl_3$): 2.63, 3.00, 3.20 (4H, 3s, aromatics); 4.15 (1H, broad s, exchanges with D_2O, OH); 6.03, 6.10 (12H, 2s, 4CH_3O); 6.07, 7.22 (4H, 2t, CH_2-CH_2). Compound 262 also gave a positive 2,4-dinitrophenylhydrazine test.

Lead tetraacetate oxidation of 256

To a stirred solution of the N-trifluoroacetyl compound 256 (0.18g, 0.39mmol) in glacial acetic acid (3cm^3) was added lead tetraacetate (0.21g, 0.47mmol). Stirring was continued for 30 mins, water (10cm^3) was added and the solution carefully basified with solid sodium bicarbonate. Extraction with chloroform (3 x 25cm^3), drying ($MgSO_4$) and removal of solvent afforded 0.16g of an oil. ν Thin film: 1750cm^{-1} ($OCOCH_3$); 1675, 1645, 1630cm^{-1} (dienone). The oil was dissolved in dichloromethane (15cm^3) to which was added trifluoroacetic acid (0.75cm^3) followed by stirring at room temperature for 2 hr. Washing of the organic solvent with saturated sodium bicarbonate solution (3 x 20cm^3), water (3 x 20cm^3) and drying ($MgSO_4$) gave 80mg of a tan solid, which was chromatographed (preparative t.l.c., ethyl acetate

petrol, 1:1) yielding 55mg of a fawn solid, m.p. 183-6° (ethyl acetate-petrol). This product was shown to be identical to 262 (infrared spectrum, R_f, nmr and mixed m.p.).

Vanadium oxytrifluoride oxidation of 256

To a solution of 256 in dichloromethane ($16cm^3$) at -10° was added a 20:1 w/w mixture of trifluoroacetic acid and trifluoroacetic anhydride ($4cm^3$). The solution was stirred during the addition of vanadium oxytrifluoride (0.31g) dissolved in the minimum volume of a 1:1 solution of ethyl acetate and trifluoroacetic acid-anhydride (20:1 w/w). After 10 mins the mixture was poured into water ($25cm^3$) and the organic phase washed with water (3 x $20cm^3$), sodium bicarbonate solution (3 x $20cm^3$) and dried over $MgSO_4$. After removal of the solvent the residual oil was triturated with ether giving a fawn solid, m.p. 175-6°, identified as the keto-imine 262.

1-(3',4',5'-Trimethoxybenzyl)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 264

The dihydroisoquinoline hydrochloride 258 (3.9g, 8mmol) was dissolved in a 1:1 mixture of glacial acetic acid-water ($40cm^3$). Zinc metal (20g) was added and the solution refluxed for 3hr., cooled and made strongly alkaline with 4M sodium hydroxide producing a white emulsion which was extracted with chloroform (3 x $100cm^3$). Washing the organic solvent with water (3 x $50cm^3$) and drying (K_2CO_3) gave after removal of the chloroform, 3.5g (97%) of the reduced product 264 as an oil which could not be crystallised and was therefore used crude in the following reaction.

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 265

Trifluoroacetic anhydride (7.4g, 0.035mol) was added dropwise to a

cooled and stirred solution of crude 264 (3.5g, 8mmol) containing triethylamine ($2cm^3$) in dry chloroform ($30cm^3$). Stirring was continued for 4 hr., the chloroform was washed with water (2 x $25cm^3$), 2M hydrochloric acid (3 x $25cm^3$) and dried ($MgSO_4$). A brown oil remained after stripping off the solvent under reduced pressure, which when triturated with cold ethanol furnished 2.0g (47%) of N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-methoxy-7-benzyloxy-1,2,3,4-tetrahydroisoquinoline 265, m.p. 155.5-6.5°, as colourless needles (ethanol). (Found: C, 64.1; H, 5.5; N, 2.2. $C_{29}H_{30}F_3NO_6$ requires C, 63.9; H, 5.5; N, 2.6%). ν_{max}^{KBr}: 1685cm^{-1} (C=O). $\tau(CDCl_3)$: 2.60 (5H, s, $PhCH_2O$); 3.35, 3.56, 3.76 (4H, 3s, aromatics); 5.00 (2H, s, $PhCH_2O$); 6.10, 6.14, 6.23 (13H, 3s, $4CH_3O$ and C-1H); 6.68, 7.35 (4H, 2t, CH_2-CH_2); 6.96 (2H, d, $PhCH_2$).

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline 263

A solution of the above benzylated material 265 (1.8g, 3mmol) in ethyl acetate ($200cm^3$) was hydrogenated in the usual manner with 5% palladised charcoal (1.5g), affording 0.8g (53%) of the title compound 263, m.p. 126-7°, as colourless crystals (ethyl acetate-petrol (b.p. 60-80°)). (Found: C, 57.3; H, 5.4; N, 2.8. $C_{22}H_{23}F_3NO_6$ requires C, 58.0; H, 5.3; N, 3.1%). ν_{max}^{KBr}: 3400cm^{-1} (O-H); 1693cm^{-1} (C=O). $\tau(CDCl_3)$: 2.93-3.26 (4H, 3s, aromatics); 4.25 (1H, broad s, exchanges with D_2O, OH); 6.03, 6.14, 6.16 (12H, 3s, $4CH_3O$); 7.14 (2H, t, CH_2-CH_2-N).

Lead tetraacetate oxidation of 263

The lead tetraacetate oxidation of compound 263 (1.0g, 2.2mmol) was carried out according to the method described for 256. A brown oil (1.15g) was obtained; ν_{max}: 1745cm^{-1} ($OCOCH_3$); 1690cm^{-1} ($NCOCF_3$); 1678, 1655, 1630cm^{-1} (dienone), which was stirred for 2 hr. in dichloromethane

($85cm^3$) and trifluoroacetic acid ($4.2cm^3$) followed by washing with saturated sodium bicarbonate (3 x $50cm^3$), water (3 x $50cm^3$) and dried over $MgSO_4$. Trituration of the residual brown oil with ice-cold ethanol gave N-trifluoroacetyl-1-hydroxy-2,9,10,11-tetramethoxyaporphine 266 (0.35g, 35%) as colourless needles (ethanol), m.p. 238-9.5°. (Found: C, 58.0; H, 4.8; N, 3.0. $C_{22}H_{22}F_3NO_6$ requires C, 58.3; H, 4.9; N, 3.1%). Found m/e =453.1411, 327.1238 (100%). (Calc. for $C_{22}H_{22}F_3NO_6$ 453.1400, 327.1233). ν_{max}^{KBr}: 3220cm^{-1} (O-H); 1680cm^{-1} (N-COCF$_3$). τ($CDCl_3$): 1.12 (1H, s, OH); 3.28, 3.34 (2H, 2s, aromatics); 6.10, 6.13, 6.23 (12H, s, 4CH_3O); 7.16 (2H, t, CH_2-CH_2-N); 8.38 (1H, s, C-6aH). UV (ethanol): 222nm (32,576); 280 (11,320); 303 (6,792).

Vanadium oxytrifluoride oxidation of 263

The oxidation was carried out using the same method and the same quantities of reagent and substrate as described for compound 256, to afford the hydroxy aporphine 266 (0.046g, 10%) as colourless crystals, m.p. 235-8°.

N-Trifluoroacetyl-1,2,9,10,11-pentamethoxyaporphine 269

Sodium hydride (0.6g, 80% dispersion in oil) was added to a solution of the hydroxyaporphine 266 (0.30g, 0.66mmol) in anhydrous DMF ($15cm^3$) and stirred for 30 mins. Iodomethane (1g, 7.0mmol) was introduced dropwise over 5 mins followed by additional stirring for 1 hr. The reaction mixture was poured into water ($50cm^3$), and extracted with chloroform (3 x $25cm^3$), the organic phase was washed with water (3 x $50cm^3$), dried ($MgSO_4$) and evaporated to dryness, furnishing colourless crystals of N-trifluoroacetyl-1,2,9,10,11-pentamethoxyaporphine 269 (0.2g, 65%), m.p. 179-80° (ethanol). (Found: C, 59.5; H, 5.3; N, 2.8. $C_{23}H_{24}F_3NO_6$

requires C, 59.1; H, 5.1; N, 3.0%). ν_{max}^{KBr}: 1685cm^{-1} ($NCOCF_3$). $\tau(CDCl_3)$: 3.37, 3.43 (2H, 2s, aromatics); 6.15, 6.24, 6.40 (15H, s, $5CH_3O$); 7.29 (2H, t, CH_2-CH_2-N). UV (ethanol): 226nm; 284nm.

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzylidene)-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 274

The title compound 274 (2.0g, 56%) was prepared from the dihydroisoquinoline 273 (3.2g, 7mmol), triethylamine (3cm^3), trifluoroacetic anhydride (9.0g, 0.043mol) in chloroform (30cm^3) using the same method as that described for 259. Crystallisation from ethanol gave colourless needles, m.p. 186-7°. (Found: C, 64.0; H, 5.2; N, 2.2. $C_{29}H_{28}F_3NO_6$ requires C, 64.0; H, 5.2; N, 2.6%). ν_{max}^{KBr}: 1700cm^{-1} ($NCOCF_3$). $\tau(CDCl_3)$: 2.72-3.45 (10H, m, aromatics and vinylic); 4.95 (2H, s, $PhCH_2O$); 6.12, 6.23 (12H, s, $4CH_3O$); 7.00 (2H, t, CH_2=CH_2-N).

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzylidene)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 271

In a similar fashion to 256 was prepared the title compound 271 in 48% yield, m.p. 187-8°, as colourless crystals (ethyl acetate-petrol). (Found: C, 57.7; H, 5.0; N, 3.0. $C_{22}H_{22}F_3NO_6$ requires C, 58.2; H, 4.9; N, 3.1%). ν_{max}^{KBr}: 3405cm^{-1} (O-H); 1703cm^{-1} ($NCOCF_3$). $\tau(CDCl_3)$: 2.84-3.35 (5H, m, aromatics and vinylic); 4.15 (1H, s, OH); 6.07, 6.17 (12H, s, $4CH_3O$); 7.03 (2H, t, CH_2=CH_2-N). Preparation of 271 directly by trifluoroacetylation of the 6-hydroxy-3,4-dihydroisoquinoline 275 (2.55g, 6mmol) gave 1.0g of a fawn coloured product, m.p. 198-9.5°, having infrared absorptions at 1810cm^{-1} and 1700cm^{-1} similar to the isomeric form 261, indicating the formation of an O,N-ditrifluoroacetyl compound. Attempts to purify this material by chromatographic methods resulted in only 271 being obtained.

Oxidation of 271 by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)

An equimolar mixture of 271 (0.5g, 1.1mmol) and DDQ in dry benzene was refluxed for 4 hr. Filtration of the cooled reaction mixture and removal of the benzene gave a black intractable mixture which was not pursued further.

1-(3',4',5'-Trimethoxybenzyl)-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 354

Reduction of the dihydroisoquinoline hydrochloride 273 (5g, 0.01mol) with zinc metal (35g) in refluxing 1:1 glacial acetic acid-water ($70cm^3$) afforded, after basification (4M sodium hydroxide), extraction with chloroform (3 x $150cm^3$), drying (K_2CO_3) and removal of solvent, 4.6g (99%) of 354 as a beige oil which could not be made to crystallise and was used directly in the following reaction.

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 277

To the above crude tetrahydroisoquinoline 354 (4.6g, 0.01mol) dissolved in dry chloroform ($30cm^3$) was added with cooling, triethylamine ($3cm^3$) and trifluoroacetic anhydride (8.9g, 0.042mol). The mixture was stirred for 4 hr., washed with water (2 x $25cm^3$), 2M hydrochloric acid (3 x $25cm^3$) and dried with $MgSO_4$. Removal of the chloroform under reduced pressure and trituration with ice cold ethanol furnished 3.0g (54%) of N-trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 277, m.p. 150.5-51.5° as colourless needles (ethanol). (Found: C, 63.5; H, 5.6; N, 2.6. $C_{29}H_{30}F_3NO_6$ requires C, 63.8; H, 5.5; N, 2.6%). ν_{max}^{KBr}: 1685cm^{-1} ($NCOCF_3$). $\tau(CDCl_3)$: 2.40-3.72 (9H, m, aromatics); 4.90 (2H, s, $PhC\underline{H}_2O$); 6.18, 6.25, 6.29 (12H, s, $4C\underline{H}_3O$); 7.36 (2H, t, $C\underline{H}_2$-CH_2-N).

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 276

Debenzylation of 277 was achieved in 92% yield using 5% Pd on charcoal with ethyl acetate as solvent, according to the procedure described for 263, giving colourless crystals of title compound 276, m.p. 181-3° (ethyl acetate-petrol (b.p. 60-80°)). (Found: C, 57.8; H, 5.4; N,3.0. $C_{22}H_{23}F_3NO_6$ requires C, 58.0; H, 5.3; N, 3.1%). ν_{max}^{KBr}: 3420cm^{-1} (O-H); 1685cm^{-1} (NCOCF$_3$). τ(CDCl$_3$): 2.89-3.37 (4H, 3s, aromatics); 4.10 (1H, broad s, exchanges with D_2O, OH); 6.08, 6.15, 6.17 (12H, 3s, 4CH$_3$O); 7.00 (2H, t, CH$_2$-CH$_2$-N).

Oxidation of 276 by 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ)

The phenolic 1-benzylisoquinoline 276 (1.0g, 2.2mmol) was refluxed in benzene (15cm^3) with DDQ (0.5g, 2.2mmol) for 1 hr. The dark brown solution was decanted from the black solid coating the reaction vessel, and evaporated to dryness giving a dark brown gum which gave 150mg of starting material on trituration with cold ethanol. The remaining liquors were chromatographed (silica gel, 30g) using ethyl acetate-petrol (2:1) as eluant, but could not be sufficiently purified to enable identification.

1-(3',4',5'-Trimethoxybenzoyl)-6-hydroxy-7-methoxy-3,4-dihydroisoquinoline 279

Anodic oxidation of the amino-acid 166 was performed according to the method of Bobbitt.[1] A solution of the acid (0.4g, 1mmol) in 0.1M sodium bicarbonate solution in 3:2 methanol-water (200cm^3) was oxidised at 280mV for 2.5 hr. The graphite felt anode was washed with methanol (3 x 100cm^3) and the combined solutions evaporated to near dryness. Addition of water (200cm^3) followed by extraction with chloroform (3 x 100cm^3) and drying ($MgSO_4$) afforded title compound 279 (0.22g, 60%), m.p. 135-6.5°, after trituration and recrystallisation from ether. (Found: C, 63.3;

H, 5.6; N, 3.5 $C_{20}H_{21}NO_6 \cdot \frac{1}{2}H_2O$ requires C, 63.2; H, 5.8; N, 3.7%). ν_{max}^{KBr}: 1670cm^{-1} (C=O). τ($CDCl_3$): 2.78-3.27 (4H, 3s, aromatics); 6.11, 6.14, 6.21 (12H, 3s, 4CH_3O); 7.26 (2H, t, CH_2-CH_2-N).

N-Trifluoroacetyl-1-(3',4',5'-trimethoxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 245

The hydrochloride salt of amino acid 166 (1.5g, 3.4mmol) suspended in dry chloroform (25cm^3) containing triethylamine (2cm^3) was cooled and stirred during the dropwise addition of just sufficient trifluoroacetic anhydride (approx. 2cm^3) to dissolve the suspended material. Stirring was continued for a further 3 hr., after which the chloroform layer was washed with water (3 x 20cm^3), 2M hydrochloric acid (3 x 20cm^3), dried over $MgSO_4$ and solvent taken off under reduced pressure, leaving a brown gum. Trituration with ether-petrol (b.p. 40-60°) resulted in a fawn coloured solid which after crystallisation from aqueous ethanol gave 1.2g (71%) of the carboxylic acid 245, m.p. 191-2.5°, as colourless needles. (Found: C, 55.2; H, 5.0; N, 2.9. $C_{23}H_{24}F_3NO_8$ requires C, 55.3; H, 4.8; N, 2.8%). Found m/e = 499.1479 (calc. for $C_{23}H_{24}F_3NO_8$ 499.1455). ν_{max}^{KBr}: 3370cm^{-1} (O-H); 1715cm^{-1} (CO_2H); 1685cm^{-1} ($NCOCF_3$). τ($CDCl_3$): 2.86, 3.34, 4.12 (4H, 3s, aromatics); 6.05 (2H, s, $PhCH_2$); 6.21, 6.40 (12H, 2s, 4CH_3O); 7.18 (2H, t, CH_2-CH_2-N).

Initial attempts to prepare the acid 245 using refluxing pyridine as the solvent gave an unexpected product, the experimental and spectroscopic details of which are presented later.

Oxidation Experiments on Acid 245

(i) Anodic Oxidation

The amido-acid 245 (0.5g, 1.0mmol) was dissolved in 0.1M sodium bicarbonate solution in 3:2 methanol-water (200cm^3), and oxidised at a graphite felt anode at 360mV for 3.7 hr. The anode was washed with methanol (3 x 100cm^3)

and the combined reaction mixture and washings reduced to low volume before dilution with water ($100cm^3$). Neutralisation of the aqueous phase with 1M hydrochloric acid was followed by extraction with chloroform (3 x $100cm^3$), drying ($MgSO_4$) and removal of solvent, giving 0.17g of a brown gum. T.l.c. showed this gum to be a multicompontent mixture in which the keto-imine 279 was shown by comparison with authentic material to be the major component. Neither starting material nor the expected unsaturated molecule 271 were apparent in the electrolysed medium. The gum was dissolved in methanol ($10cm^3$) and stirred overnight with a freshly prepared ethereal solution of diazomethane. The solution was evaporated to dryness, dissolved in ethanol and hydrogenated using platinum oxide (50mg) for 6 hr. T.l.c. of this reaction mixture in three different solvent systems, viz., chloroform-methanol (50:1), ethyl acetate-petrol (2:1) and benzene-acetone (25:1) exhibited no spot corresponding to the aporphine 269.

The above procedure was repeated using as the solvent for electrolysis, a 9:1 mixture of acetonitrile-water ($200cm^3$) containing sodium bicarbonate (4g) and tetraethylammonium perchlorate (4g). Oxidation at 270mV for 2.75 hr. gave 0.3g of brown oil which was shown by t.l.c. both before and after methylation-reduction to be identical with the previous anodic oxidation.

(ii) Enzymic Oxidation

Prior to use both laccase and horseradish peroxidase solutions were assayed by the methods previously described. To a solution of the carboxylic acid 245 (0.5g, 1.0mmol) in phosphate buffer (pH6, $100cm^3$) was added just sufficient ethanol to dissolve the solid material. To half of this solution was added laccase (six additions of $5cm^3$ over

3hr.). The mixture was left overnight, extracted with chloroform (3 x 100cm^3), dried ($MgSO_4$) and evaporated to dryness affording a brown gum (0.18g). To the remaining half of the acid solution was added peroxidase solution (0.1g in 100cm^3 pH6 phosphate buffer, 2cm^3) and 3% hydrogen peroxide (2cm^3). The mixture was allowed to stand overnight and worked up as for the laccase test solution.

Comparative t.l.c. (ethyl acetate-petrol (2:1)) showed a substantial amount of 279 in both laccase- and peroxidase-induced reactions, the former also showed the presence of the 3,4-dihydroisoquinoline 172, as well as having much less polymeric base line material. No significant amounts of material were evident in the aqueous residues from either enzyme reaction. Methylation (diazomethane) and catalytic reduction (platinum oxide) of the fraction soluble in organic solvent gave a residue which failed to show the presence of any N-trifluoroacetyl-1,2,9,10,11-pentamethoxyaporphine 269. An experimental blank containing only acid and buffer showed only unreacted starting material when allowed to stand for the same period under identical conditions.

(iii) Chemical Oxidations

(a) Potassium ferricyanide

Isoquinoline-1-carboxylic acid 245 (0.5g, 1.0mmol) and potassium ferricyanide (0.33g, 1mmol) were stirred together for 5hr. in a two phase 5% sodium bicarbonate-chloroform mixture (1:1, 20cm^3). Neutralisation with 1M hydrochloric acid followed by extraction into chloroform (3 x 25cm^3), drying ($MgSO_4$) and removal of solvent gave 0.32g of a dark brown product. T.l.c. (ethyl acetate-petrol, 2:1) showed the presence of a small amount of starting acid and base line material as well as the major product identified by subsequent preparative t.l.c. as the keto-imine 279.

(b) Cuprammonium complex

The attempted oxidation of 245 (0.1g, 0.2mmol) by an aqueous cuprammonium species (formed from equal volumes of Fehling's A and B solutions, $10cm^3$) and chloroform ($10cm^3$) gave upon work up in the previously described manner, only unreacted starting material.

(c) Manganese dioxide

Active manganese dioxide (0.2g) and acid 245 (0.1g, 0.2mmol) in dry benzene were refluxed for 4 hr. Filtration of the hot suspension followed by removal of the solvent gave 70mg of product having the same R_f and infrared spectrum as 279, as the sole product.

(d) D.D.Q.

Equimolar amounts of acid 245 (0.25g, 0.5mmol) and DDQ (0.11g, 0.5mmol) were refluxed in dry toluene for 5 hr., after which the solution was cooled and filtered. Removal of the toluene gave a dark brown product (0.17g) shown by t.l.c. (ethyl acetate-petrol, 2:1) to contain a significant amount of 279 and residual reagent. Two minor products having very similar R_f's to 279 were also observed, but attempts to isolate either by preparative t.l.c. proved unsuccessful.

WORK DESCRIBED IN CHAPTER FOUR

2-Benzyloxybenzaldehyde 294

To a stirred solution of sodium (20g) in ethanol (600cm^3) was added salicylaldehyde (100g, 0.82mol) and benzyl chloride (130g, 1.03mol). This mixture was refluxed for 6 hr. after which it was filtered hot, cooled and reduced in volume to approximately 200cm^3 affording on further cooling colourless crystals of title compound 294 (122g, 70%), m.p. 44.5-46.5 (lit 46°)[332] from ethanol. ν_{max}^{KBr}: 1685cm^{-1} (C=O). $\tau(CDCl_3)$: -0.48 (1H, s, CHO); 2.12-3.18 (9H, m, aromatics); 4.90 (2H, s, $PhCH_2O$).

2-Phenyl-4-(2'-benzyloxybenzylidene)-2-oxazolin-5-one 299

The above benzaldehyde (50g, 0.236mol), hippuric acid (50g), anhydrous sodium acetate (25g) and acetic anhydride (100cm^3) were heated together on a steam bath for 2.5 hr. The mixture was stirred frequently and upon cooling overnight at 4° gave yellow needles which were filtered off, washed with ethanol and ether, and air dried to give 299 (77.1g, 92%), m.p. 164-5° (lit 164-5°).[332] ν_{max}^{KBr}: 1800cm^{-1} (C=O); 1650cm^{-1} (C=C). $\tau(CDCl_3)$: 1.72-3.10 (14H, m, aromatics); 2.05 (1H, s, vinylic); 4.84 (2H, s, $PhCH_2O$).

2-Thio-oxazolid-4-one 355

Potassium cyanide (65g) and potassium thiocyanate (97g) were mixed together in water (20cm^3). Formaldehyde solution (31cm^3, 38%) was added to the resultant slurry, followed by the dropwise addition of conc. hydrochloric acid (75cm^3) over 30 mins, the mixture was allowed to stand overnight and was filtered. The filtrate was refluxed for 2hr., cooled, extracted with ether (3 x 50cm^3), dried ($MgSO_4$) and evaporated to dryness, furnishing after recrystallisation (benzene) and charcoal

treatment, colourless crystals of 2-thio-oxazolid-4-one 355, m.p. 107-8° (lit 113°).[372] ν_{max}^{KBr}: 1720 (C=O, lactam); 1655cm^{-1} (C=C).

5-(2'-Benzyloxybenzylidene)-2-thio-oxazolid-4-one 298

A mixture of 2-thio-oxazolid-4-one 355 (2.5g, 0.021mol), 2-benzyloxybenzaldehyde 294 (5.2g, 0.025mol), sodium acetate (2.5g) and glacial acetic acid (12.5cm^3) were brought to reflux over 1 hr. Title compound 298 (3.7g, 48%) crystallised out on cooling, and was recrystallised from glacial acetic acid to afford yellow crystals, m.p. 209-10°. (Found: C, 65.6; H, 4.6; N, 4.5. $C_{17}H_{13}NO_3S$ requires C, 65.6; H, 4.2; N, 4.5%). τ($CDCl_3$): 1.76-3.04 (10H, m, aromatics and vinylic); 4.83 (2H, s, $PhC\underline{H}_2O$).

2-Benzyloxyphenylpyruvic Acid 296

The azlactone 299 (50g, 0.141mol) was refluxed for 5 hr. in 4M sodium hydroxide solution (300cm^3) and poured into barium chloride solution (84g, $BaCl_2 \cdot 2H_2O$ in 600cm^3 water). The resulting barium salt was filtered off and heated at 40-50° in 2M hydrochloric acid (500cm^3) for 30 mins. to give white crystals of the pyruvic acid 296 (14.8g, 78%), m.p. 123-123.5° (lit 125°)[332] from benzene-petrol (b.p. 80-100°). ν_{max}^{KBr}: 3450cm^{-1} (O-H, Enol); 1700cm^{-1} (CO_2H); 1660cm^{-1} (C=C). τ($CDCl_3$): 0.95 (1H, broad s, exchanges with D_2O, $CO_2\underline{H}$); 2.53-3.03 (9H, m, aromatics); 4.91 (2H, s, $PhC\underline{H}_2O$); 5.73 (2H, s, $PhC\underline{H}_2$). 2-Benzyloxy phenylpyruvic acid was found to be air sensitive and was therefore kept in a sealed tube at 4°.

Preparation of this same α-keto acid from compound 298 (1g, 3.0mmol) was achieved by refluxing in 1M sodium hydroxide solution (25cm^3) for 2 hr., acidifying with 2M hydrochloric acid and extraction with ether (2 x 25cm^3). Drying ($MgSO_4$) and removal of the ether gave 0.5g (57%)

of 2-benzyloxyphenylpyruvic acid 296, m.p. 114·5-118°, which had the same R_f (ethanol-chloroform-ammonia, 5:3:1.5) as that prepared by the previous method.

1-(2'-Benzyloxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 297

2-Benzyloxyphenylpyruvic acid 296 (3.8g, 0.014mol) was dissolved in 4M sodium hydroxide solution ($50cm^3$) and saturated with carbon dioxide. To this was added 3-hydroxy-4-methoxy-β-phenylethylamine hydrochloride 337 (3.2g, 0.016mol) dissolved in water ($20cm^3$) and the mixture heated on a steam bath for 1hr. The resulting solid was filtered off, washed with water ($500cm^3$), meths ($100cm^3$) and air dried to give the title amino acid 297 (5.3g, 90%), which was recrystallised immediately from methanol-conc. hydrochloric acid-ether to give the hydrochloride salt, m.p. 239-40°, as colourless crystals. (Found: C, 62.3; H, 5.7; N, 2.7. $C_{25}H_{26}NO_5Cl$ requires C, 65.9; H, 5.7; N, 3.1%). ν_{max}^{KBr}: 3450cm^{-1} (O-H); 1725cm^{-1} (CO_2H). $\tau(D_2O)$: 2.82-3.51 (11H, m, aromatics); 4.95 (2H, s, $PhCH_2O$); 6.13 (3H, s, CH_3O); 7.04 (2H, t, CH_2-CH_2-N).

N-Trifluoroacetyl-1-(2'-benzyloxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 300

A suspension of amino acid hydrochloride 297 (1.9g, 4.0mmol) in chloroform ($25cm^3$) containing triethylamine ($2cm^3$) was stirred and cooled during the addition of trifluoroacetic anhydride (2.7g, 0.013mol). After stirring for 3 hr. the solution was washed with water (3 x $20cm^3$), 2M hydrochloric acid (3 x $20cm^3$), dried ($MgSO_4$) and evaporated to dryness. Trituration of the resulting oil with ice cold ethanol gave title compound 300 (1.0g, 47%), m.p. 168-70°, as colourless needles (aqueous ethanol). (Found: C, 63.3; H, 5.0; N, 2.5. $C_{27}H_{24}F_3NO_6$ requires C, 62.9; H, 4.7; N, 2.7%). Found m/e = 515.1588 (Calc. $C_{27}H_{24}F_3NO_6$ requires 515.1557. ν_{max}^{KBr}: 1715cm^{-1}

(CO_2H); 1705cm^{-1} ($NCOCF_3$). τ(DMSO): 0.6(1H, broad s, $CO_2\underline{H}$); 2.66-3.50 (11H, m, aromatics); 5.05 (2H, s, $PhC\underline{H}_2O$); 6.27 (3H, s, $C\underline{H}_3O$).

1-(2'-Hydroxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid hydrochloride 301

The hydrochloride of amino acid 297 (1.9g, 4.0mmol) was O-debenzylated by refluxing for 2 hr. in a 1:1 mixture of conc. hydrochloric acid-acetic acid (50cm^3). The solvents were distilled off under reduced pressure to give a brown oil which on trituration with acetone furnished a colourless solid. Crystallisation (aqueous ethanol) afforded the hydrochloride of 1-(2'-hydroxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 301 (1.4g, 92%) as colourless crystals, m.p. 262-4° (d). (Found: C, 58.6; H, 5.6; N, 3.8. $C_{18}H_{20}NO_5Cl$ requires C, 59.1; H, 5.5; N, 3.8%). ν_{max}^{KBr}: 3180cm^{-1} (O-H); 1710cm^{-1} (CO_2H). $\tau(D_2O)$: 2.83-3.54 (6H, m, aromatics); 6.16 (3H, s, $C\underline{H}_3O$).

Lactone of N-formyl-1-(2'-hydroxybenzyl)-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 302

The amino acid 301 (2.0g, 6.1mmol) was dissolved in freshly prepared acetic-formic anhydride (15cm^3) made according to the method of Sheehan.[310] The solution was diluted with ether (50cm^3) and stirred at room temperature overnight. Evaporation to dryness, followed by trituration with benzene-petrol (b.p. 80-100°) gave 0.4g of a white amorphous solid, shown by t.l.c. (benzene-acetone, 3:1; silica gel) to contain at least three constituents. Preparative t.l.c. (benzene-acetone, 5:1, silica gel) on 150mg of this solid produced the δ-lactone 302 (65mg), m.p. 105-8°, as a colourless solid (aqueous ethanol). (Found: C, 66.8; H, 5.1; N, 4.1. $C_{19}H_{17}NO_5$ requires C, 67.3; H, 5.0; N, 4.1%). ν_{max}^{KBr}: 3350cm^{-1} (O-H); 1755cm^{-1} (lactone); 1655cm^{-1} (NCHO). $\tau(CDCl_3)$: 2.13 (1H, s, C$\underline{H}$O); 2.88-3.75 (6H, m, aromatics); 4.15 (1H, broad s, exchanges

with D_2O, OH); 6.61 (3H, s, CH_3O); 6.97 (2H, t, CH_2-CH_2-N).

N-Benzoyl-1-cyano-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 291

Benzoyl chloride (4.8g, 0.035mol) was added dropwise over 30 mins. to a stirred two phase mixture of dihydroisoquinoline 292 (3.0g, 0.011mol) dissolved in dichloromethane ($25cm^3$) and potassium cyanide (3.5g, 0.054 mol) dissolved in water ($10cm^3$). Stirring was continued for 3 hr., followed by washing of the dichloromethane layer with 2M sodium hydroxide (3 x $25cm^3$), 2M hydrochloric acid (3 x $25cm^3$) and water (3 x $25cm^3$). Drying ($MgSO_4$) and removal of solvent afforded a brown oil which upon trituration with methanol gave colourless needles of N-benzoyl-1-cyano-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 291 (2.7g, 60%), m.p. 139-41° (methanol). (Found: C, 75.2; H, 5.8; N, 7.0. $C_{25}H_{22}N_2O_3$ requires C, 75.4; H, 5.5; N, 7.0%). ν_{max}^{KBr}: 1655cm^{-1} (C=O). $\tau(CDCl_3)$: 2.13-3.25 (12H, m, aromatics); 4.82 (2H, s, $PhCH_2O$); 6.05 (3H, s, CH_3O); 7.22 (2H, t, CH_2-CH_2-N).

2-Benzyloxybenzyl alcohol 295

Sodium borohydride (5g) was added in small portions to a stirred solution of 2-benzyloxybenzaldehyde 294 (10g, 0.047mol) in ethanol and stirred overnight at room temperature. The residue obtained on removal of the ethanol was dissolved in water ($100cm^3$), extracted with ether (3 x $50cm^3$), dried ($MgSO_4$) and evaporated to dryness affording a yellow oil which was distilled (b.p. 160-2° at 0.5mm Hg) to give 295 (0.75g, 74%), m.p. 36-7° (lit 37°)[373] as colourless crystals. ν_{max}^{KBr}: 3400cm^{-1} (O-H). $\tau(CDCl_3)$: 2.68-3.27 (9H, m, aromatics); 5.10 (2H, s, $PhCH_2O$); 5.35 (2H, s, CH_2OH); 6.91 (1H, s, CH_2OH).

2-Benzyloxybenzyl chloride 293

A mixture of thionyl chloride ($20cm^3$), benzene ($50cm^3$) and 2-benzyloxy-

benzyl alcohol 295 (10g, 0.047mol) was refluxed for 5 hr. The benzene and excess thionyl chloride were distilled off at atmospheric pressure leaving a pale green oil (9.9g, 91%) which was used crude in the following reaction.

N-Benzoyl-1-cyano-1-(2'-benzyloxybenzyl)-6-benzyloxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline 290

The Reissert compound 291 (2.7g, 6.8mmol) was dissolved in dry DMF ($25cm^3$) and to this stirred solution was added sodium hydride (0.38g, 50% dispersion in oil), producing the expected deep red colour of the Reissert anion. The dropwise addition of crude 2-benzyloxybenzyl chloride 293 (3.3g, 0.014mol) dissolved in DMF ($10cm^3$) gradually removed the red colouration to give a pale yellow solution which after a further 15 hr. stirring at room temperature was poured on to ice-water ($200cm^3$) and extracted with chloroform (3 x $50cm^3$). The organic phase was washed with 2M hydrochloric acid (2 x $50cm^3$), water (3 x $50cm^3$), 2M sodium hydroxide (2 x $50cm^3$) and dried ($MgSO_4$). A brown solid (0.9g, m.p. 158-167°) was obtained after removing the chloroform and trituration with ether, which could not be purified sufficiently for identification.

3-Methoxy-4-hydroxy-β-phenylethylamine hydrochloride 307

Catalytic hydrogenation of the benzyloxy-amine 347 using 5% palladium on charcoal in ethanol gave the title compound 307, m.p. 209-10° (lit 212-3°) in 92% yield. ν_{max}^{KBr}: 3150cm^{-1} (O-H); 2640, 2560, 2450cm^{-1} (salt bands).

2-Benzyloxyphenylacetic acid 306

Potassium hydroxide (12g) dissolved in water ($20cm^3$) was added to a stirred solution of 2-hydroxyphenylacetic acid (15g, 0.099mol) and benzyl chloride (26.4g, 0.21mol) in ethanol ($150cm^3$). The mixture was refluxed

for 4 hr., evaporated to near dryness and the residue was partitioned between chloroform ($100cm^3$) and water ($50cm^3$). The chloroform layer was washed twice more with water ($50cm^3$) and dried over $MgSO_4$. Removal of solvent gave a yellow solid (12g) m.p. 69-72° which t.l.c. (chloroform-silica gel) showed to contain two components, a major product of R_f 0.68 and a minor component of R_f 0.10. Column chromatography using chloroform as eluant gave both compounds in a pure state. The major constituent (7.5g), m.p. 75-6° (petrol (b.p. 60-80°)) did not react with saturated sodium bicarbonate solution and was identified as benzyl 2-benzyloxyphenylacetate 305. (Found: C, 79.8; H, 6.2. $C_{22}H_{20}O_3$ requires C, 79.5; H, 6.0%). ν_{max}^{KBr}: 1735cm^{-1} (C=O, ester). $\tau(CDCl_3)$: 2.72-3.20 (14H, m, aromatics); 4.96 (2H, s, $PhC\underline{H}_2O$); 5.01 (2H, s, $PhC\underline{H}_2OCO$); 6.29 (2H, s, $PhC\underline{H}_2CO_2$). The minor product did react with saturated sodium bicarbonate solution and was found to be the desired acid 306 (4.1g, 17%), m.p. 92.5-5.5° (lit 92-4°).[374] ν_{max}^{KBr}: 1710cm^{-1} (CO_2H). $\tau(CDCl_3)$: -1.84 (1H, broad s, exchanges with D_2O, $CO_2\underline{H}$); 2.75-3.28 (9H, m, aromatics); 5.04 (2H, s, $PhC\underline{H}_2O$); 6.37 (2H, s, $PhC\underline{H}_2CO_2H$).

Hydrolysis of the benzyl ester 305 (8.4g, 0.025mol) with refluxing 4M sodium hydroxide solution ($150cm^3$) for 6 hr. afforded after cooling and acidification with conc. hydrochloric acid, 2-benzyloxyphenylacetic acid 306 (3.8g, 62%), m.p. 93-5°, as colourless crystals.

3-Methoxy-4-hydroxy-5-bromo-β-phenylethylamine hydrochloride 308

A solution of bromine (7.6g, 0.047mol) in 1,4-dioxan ($30cm^3$) was added over 20 mins. to a suspension of 3-methoxy-4-hydroxy-β-phenylethylamine hydrochloride 307 (6.2g, 0.030mol) in glacial acetic acid ($40cm^3$) maintained at a temperature of 70°. After stirring for 1.5 hr. the solvents were distilled off under reduced pressure to give an orange oil

which upon trituration with ether gave the title compound 308 (6.3g, 73%), m.p. 207-12° (lit 215-7°)[336] as colourless crystals (ethanol-ether). ν_{max}^{KBr}: 3430cm^{-1} (O-H); 2750, 2600, 2550, 2420cm^{-1} (salt bands).

N-(3-Methoxy-4-hydroxy-5-bromo-β phenylethyl)-2-benzyloxyphenylacetamide 309

The bromo-amine 308 (2.9g, 0.012mol) was dissolved in water and the solution made basic with ammonia solution. A white solid of the resultant free base was formed which was filtered off and air dried. This free base (2.4g, 0.011mol) and 2-benzyloxyphenylacetic acid 306 (2.6g, 0.010mol) were heated together at 180° for 1 hr. in decalin (30cm^3) under nitrogen. After decanting off the decalin the residue was washed with petrol (3 x 50cm^3), dissolved in chloroform (100cm^3), washed with water (2 x 75cm^3), 2M hydrochloric acid (3 x 75cm^3), 2M sodium bicarbonate solution (3 x 75cm^3) and dried ($MgSO_4$). The solvent was removed under reduced pressure affording a black gum which was passed down a silica-gel column (150g) using chloroform as eluant, to give the title amide 309 (1.4g, 31%), m.p. 124-6° as a fawn coloured solid. (Found: C, 61.5; H, 5.0; N, 2.9. $C_{24}H_{24}BrNO_4$ requires C, 61.3; H, 5.1; N, 3.0%). ν_{max}^{KBr}: 3350cm^{-1} (N-H); 1650cm^{-1} (C=O). τ($CDCl_3$): 2.66-3.49 (11H, m, aromatics); 4.09 (1H, broad s, NH); 5.05 (2H, s, $PhCH_2O$); 6.32 (3H, s, CH_3O); 6.42 (2H, s, $PhCH_2CO$); 6.61, 7.16 (4H, 2t, CH_2-CH_2).

3-Hydroxy-4,5-dimethoxybenzoic acid 311

The synthetic method used to prepare 311 was that reported by Battersby et al.[337] 3,4,5-Trihydroxybenzoic acid monohydrate (50g, 0.266mol) gave as the major product from this reaction, 3,4,5-trimethoxybenzoic acid (16.0g), m.p. 158-60° (lit 157-60°).[376] In addition, compound 311 (4.1g), m.p. 187-90° (lit 188-90°)[337] was obtained as colourless crystals (water) from the acidified aqueous washings. Found m/e = 198.0530. $C_9H_{10}O_5$

requires 198.0529. ν_{max}^{KBr}: 3520cm^{-1} (O-H); 1695cm^{-1} (CO_2H). τ(DMSO): 2.85, 2.90 (2H, 2s, aromatics); 6.18, 6.24 (6H, 2s, $2CH_3O$).

3-Benzyloxy-4,5-dimethoxybenzoic acid 312

A mixture of 3-hydroxy-4,5-dimethoxybenzoic acid 311 (2.5g, 0.013mol), benzyl chloride (5.5g, 0.043mol) and potassium hydroxide (2g in 5cm^3 water) dissolved in ethanol (50cm^3) was refluxed for 4 hr. The solvent was removed leaving a brown oil which was dissolved in chloroform (50cm^3), washed with water (3 x 50cm^3) and dried ($MgSO_4$). Evaporation of the chloroform gave on trituration with petrol (b.p. 60-80°) and crystallisation from aqueous ethanol, colourless crystals of 3-benzyloxy-4,5-dimethoxybenzoic acid 312 (0.8g, 22%), m.p. 169-73° (lit 176°).[375] ν_{max}^{KBr}: 1685cm^{-1} (CO_2H). τ($CDCl_3$): 1.60 (1H, broad s, exchanges with D_2O, $CO_2\underline{H}$); 2.66 (7H, s, aromatics); 4.90 (2H, s, $PhC\underline{H}_2O$); 6.12 (6H, s, $2C\underline{H}_3O$).

Oxidation experiments on acid 300

(i) Anodic oxidation

Electrochemical oxidation of 300 (0.25g, 0.5mmol) was carried out at 280mV (against S.C.E.) for 2 hr. in a 0.1M sodium bicarbonate solution in aqueous methanol (200cm^3). The organic solvent was removed under reduced pressure and the residual aqueous solution neutralised with 2M hydrochloric acid. Extraction with chloroform (3 x 100cm^3), drying ($MgSO_4$) and removal of the solvent gave a brown oil which upon trituration with ether gave 1-(2'-benzyloxybenzoyl)-6-hydroxy-7-methoxy-3,4-dihydroisoquinoline 313, m.p. 113.5-4.5° as pale yellow crystals, (ether-petrol (b.p. 40-60°)). (Found: C, 74.7; H, 5.9; N, 3.3. $C_{24}H_{21}NO_4$ requires C, 74.4; H, 5.4; N, 3.6%). ν_{max}^{KBr}: 1665cm^{-1} (C=O). τ($CDCl_3$): 2.79-3.45 (11H, m, aromatics); 5.09 (2H, s, $PhC\underline{H}_2O$); 6.27 (3H, s, $C\underline{H}_3O$); 6.40, 7.61 (4H, 2t, $C\underline{H}_2$-$C\underline{H}_2$).

(ii) Enzymic oxidation

The enzyme-induced oxidations of acid 300 (0.14g, 0.3mmol) with laccase and horseradish peroxidase were performed in an identical manner to those described for acid 245 (see page 172). Both enzyme reactions gave as the sole product the keto-imine compound 313, identified by comparison of its R_f and infrared spectrum, with authentic material.

WORK DESCRIBED IN CHAPTER FIVE

5.1 Oxidation Studies on Acid 315

5.1.1 N-Trifluoroacetyl-1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 315

Trifluoroacetic anhydride (10.4g, 0.05mol) was added dropwise to a cooled suspension of 1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 164 (3.0g, 0.013mol) in chloroform ($40cm^3$) containing triethylamine ($3cm^3$). The resulting solution was stirred at room temperature for 3 hr., after which it was washed with water (3 x $50cm^3$), 2M hydrochloric acid (3 x $50cm^3$) and dried ($MgSO_4$). Removal of the chloroform and trituration with ice cold ethanol furnished N-trifluoroacetyl-1-methyl-6-hydroxy-7-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 315 (2.2g, 52%), m.p. 170-3° as colourless crystals (aqueous ethanol). (Found: C, 50.5; H, 4.4; N, 4.2. $C_{14}H_{14}F_3NO_5$ requires C, 50.4; H, 4.2; N, 4.2%). Found m/e = 333.0813 (Calc. for $C_{14}H_{14}F_3NO_5$ 333.0824). ν_{max}^{KBr}: $3200cm^{-1}$ (O-H); $1730cm^{-1}$ (CO_2H); $1685cm^{-1}$ ($COCF_3$). $\tau(CDCl_3)$: 3.16, 3.35 (2H, 2s, aromatics); 4.32 (1H, broad s, exchanges with D_2O, O<u>H</u>); 6.17 (3H, s, C<u>H</u>$_3$O); 8.18 (3H, s, C<u>H</u>$_3$).

2,2'-Dihydroxy-5,5'-di-tert-butylbiphenyl 316

4-Tert-butylphenol (1g, 6.7mmol) was dissolved in methanol ($50cm^3$) in which sodium metal (0.3g, 0.013mol) had previously been dissolved. This solution was evaporated to dryness and the residue taken up in water ($20cm^3$), and added to a solution of tetraethylammonium perchlorate (6g) in acetonitrile ($400cm^3$). Electrochemical oxidation of this solution with a carbon felt anode and a platinum cathode was carried out at 300mV for 4 hr., after which time the current passing was virtually zero. The electrolysed solution was acidified and the carbon electrode washed with

methanol (3 x 100cm^3). The combined solutions were then evaporated to dryness, and the residue dissolved in a 1:1 chloroform-water mixture (100cm^3). The organic solvent was washed with water (3 x 50cm^3), dried ($MgSO_4$) and evaporated, leaving a brown oil, which upon trituration with petrol (b.p. 40-60°) gave the title compound 316 (0.3g, 30%), m.p. 207-9° (lit 207-8°)[338] as colourless crystals (isooctane). ν_{max}^{KBr}: 3250cm^{-1} (O-H). $\tau(CDCl_3)$: 3.18-3.49 (6H, m, aromatics); 8.65 (18H, s, 2C($\underline{CH}_3$)$_3$).

The bis-toluene-4-sulphonyl derivative 317 was prepared by refluxing 316 (0.1g, 0.3mmol) with toluene-4-sulphonyl chloride (0.13g, 0.7mmol) in acetone (2cm^3) containing triethylamine (0.2cm^3) for 50 mins. Pouring the reaction mixture into 2M hydrochloric acid (10cm^3) gave a beige solid, which after recrystallisation from meths, afforded 317, m.p. 159-61°. (Found: C, 67.2; H, 6.3. $C_{34}H_{38}O_6S_2$ requires C, 67.3; H, 6.3%). $\tau(CDCl_3)$: 2.98-3.36 (14H, m, aromatics); 7.64 (6H, s, 2$\underline{CH}_3$); 8.71 (18H, s, 2C($\underline{CH}_3$)$_3$).

5.1.2. Oxidation of 315

(a) Anodic oxidation

Acid 315 (0.3g, 0.9mmol) was anodically oxidised in 0.1M sodium bicarbonate solution in methanol-water (3:2, 250cm^3) at 320mV for 2 hr. The pH of the reaction mixture was then adjusted to pH7 using 2M hydrochloric acid and the volatile components removed under reduced pressure. The residue was dissolved in a 1:1 mixture of chloroform and water (50cm^3), the aqueous phase was extracted with chloroform (3 x 25cm^3), the combined extracts dried ($MgSO_4$) and reduced to low volume. T.l.c. (chloroform-methanol, 5:1, silica gel) showed a complete absence of

any products. The aqueous phase was evaporated to dryness under reduced pressure and the residue triturated with chloroform. T.l.c. of this solution showed a single product which had the same blue fluorescence and infrared spectrum as the 3,4-dihydroisoquinoline 170.

Repetition of this electrolysis in the presence of 4-tert-butylphenol (0.6g, 4.0mmol) gave identical results, with the initial chloroform extract showing only the unreacted phenol.

(b) Enzymic oxidation

A stock solution of acid 315 (0.8g, 0.024mol) in pH6 phosphate buffer (200cm^3) containing just sufficient ethanol to dissolve the suspended acid (approx 20cm^3), was prepared. This solution was divided into four equal portions, and to the aliquots were added the following:-

(i) laccase solution (15cm^3)

(ii) laccase solution (15cm^3) and 4-tertiary-butylphenol (0.2g, 1.3mmol)

(iii) horseradish peroxidase solution (50mg in 50cm^3 water, 1cm^3) and 3% hydrogen peroxide (1cm^3)

(iv) as (iii) plus 4-tertiary-butylphenol (0.2g).

All four solutions were left at room temperature for 24 hr., extracted with chloroform and dried ($MgSO_4$). The remaining aqueous phase was evaporated to dryness and the resulting residue dissolved in methanol. Each solution was examined by t.l.c. (chloroform-methanol, 10:1, silica gel).

The chloroform extracts from (i) and (iii) failed to show any material detectable by iodine vapour or under u.v. light. The only chloroform soluble product from (ii) was unreacted 4-tertiary-butylphenol. Similarly (iv) showed a preponderance of this same phenol in addition to some base-line material.

The methanol solutions from (i)-(iv) all showed the presence of a component having the same R_f and characteristic blue fluorescence as the 3,4-dihydroisoquinoline 170. Solutions derived from peroxidase media exhibited far more polymeric base line material than their laccase counterparts.

5.2 Attempted Synthesis of β,β-Dimethyl-3,4,5-Trimethoxyphenylpyruvic Acid 318

3,4,5-Trimethoxybenzoic acid 356

Gallic acid (100g, 0.59mol) was dissolved in 4M sodium hydroxide solution ($1dm^3$) and stirred during the addition of dimethyl sulphate (178g) over 20 mins., the temperature being kept between 30-35°. Additional dimethyl sulphate (178g) was introduced and the mixture refluxed for 2 hr., followed by the addition of 10M sodium hydroxide solution ($100cm^3$) and reflux was continued for a further 2 hr. The reaction mixture was cooled and made acidic with conc. hydrochloric acid resulting in the precipitation of a light brown solid, which was filtered off and recrystallised from aqueous ethanol affording acid 356 (101g, 81%) as white platelets, m.p. 158-61° (lit 157-60°).[376] ν_{max}^{KBr}: $1685cm^{-1}$ (CO_2H). $\tau(CDCl_3)$: -2.43 (1H, s, exchanges with D_2O, $CO_2\underline{H}$); 2.66 (2H, s, aromatics); 6.10 (9H, s, $3C\underline{H}_3O$).

3,4,5-Trimethoxybenzoyl chloride 322

The carboxylic acid 356 (10g, 0.047mol) was refluxed with thionyl chloride ($100cm^3$) containing 5 drops of pyridine for 4 hr., following which the excess thionyl chloride was distilled off to leave a brown oil, giving upon trituration with petrol (b.p. 40-60°) the acid chloride 322 (8.1g, 74%), m.p. 75.5-79.0° (lit 77-8°),[376] as colourless crystals from chloroform-petrol (b.p. 60-80°). ν_{max}^{KBr}: $1760cm^{-1}$ (C=O). $\tau(CDCl_3)$: 2.67 (2H, s, aromatics); 6.06, 6.09 (9H, 2s, $3C\underline{H}_3O$).

Methyl 3,4,5-trimethoxybenzoate 320

A solution of 3,4,5-trimethoxybenzoic acid 356 (49g, 0.23mol) in methanol ($300cm^3$) containing conc. hydrochloric acid ($10cm^3$) was refluxed for 6 hr. T.l.c. (chloroform, silica gel) showed only a small amount of starting material in the reaction mixture, which on

cooling furnished white needles of title compound 320 (39.8g, 76%), m.p. 83-4° (lit 82-4°).[377] ν_{max}^{KBr}: 1720cm^{-1} (C=O). τ($CDCl_3$): 2.76 (2H, s, aromatics); 6.13 (12H, s, $3CH_3O$ and CO_2CH_3).

α,α-Dimethyl-3,4,5-trimethoxybenzyl alcohol 319

Methyl lithium (1.3M solution in ether) was assayed according to the method of Kofron,[340] and added in portions (4 x 50cm^3) by syringe under nitrogen to a stirred solution of ester 320 (16.8g, 0.074mol) in dry ether (200cm^3). The mixture was stirred for 1 hr., poured on to crushed ice (100g), extracted with ether (3 x 100cm^3) and the combined extracts dried over $MgSO_4$. Evaporation of the ether left a colourless oil which eventually crystallised from petrol (b.p. 40-60°) giving α,α-dimethyl-3,4,5-trimethoxybenzyl alcohol 319 (14.3g, 85%), m.p. 54-6° as colourless needles. (Found: C, 63.5; H, 8.0. $C_{12}H_{18}O_4$ requires C, 63.7; H, 8.0%). ν_{max}^{KBr}: 3500cm^{-1} (O-H). τ($CDCl_3$): 3.33 (2H, s, aromatics); 6.18, 6.20 (9H, 2s, $3CH_3O$); 7.22 (1H, s, OH); 8.46 (6H, s, $2CH_3$).

The use of 3,4,5-trimethoxyacetophenone (10g, 0.048mol) instead of the ester, with methyl lithium (1.3M solution in ether (60cm^3)), gave by the same experimental procedure, 5.5g (51%) of alcohol 319, m.p. 50.5-3°.

Originally the above alcohol was prepared in inferior yields using methyl magnesium iodide, according to the following method. Magnesium turnings (3.6g, 0.15mol) were added to dry ether (75cm^3) to which iodomethane (20.9g, 0.15mol) was introduced dropwise with stirring at such a rate as to bring about a steady rate of reflux. To the freshly prepared methyl magnesium iodide was added methyl 3,4,5-trimethoxybenzoate 320 (15.0g, 0.066mol) dissolved in dry tetrahydrofuran (50cm^3). Stirring of the green coloured semi-solid mixture was continued for 12 hr.,

followed by pouring into 1M ammonium chloride solution ($100cm^3$) and extraction with ether ($3 \times 75cm^3$). After drying (K_2CO_3) and removing the solvent, 12.2g of a yellow oil was obtained which t.l.c. (chloroform, silica gel) showed to contain two components. The major product ($R_f = 0.27$) and minor constituent ($R_f = 0.63$) were separated by column chromatography (300g, silica gel) using chloroform as eluant. The major component (10.6g) was identified as starting material, whereas the slower running compound (1.05g, 7%) had an infrared spectrum identical to that of the genuine α,α-dimethyl alcohol 319 prepared *via* the methyl lithium route. Variations on this grignard reaction were attempted using either wholly tetrahydrofuran as the solvent or using acid chloride 322 in place of ester 320; in each case low yields of the required alcohol (8 and 22% respectively) were obtained.

Attempted synthesis of α,α-dimethyl-3,4,5-trimethoxybenzyl halide

Attempts to prepare the corresponding halide (either bromide or chloride) from alcohol 319 were carried out by the following methods.

(a) α,α-Dimethyl-3,4,5-trimethoxybenzyl alcohol 319 (3.6g, 0.016mol) dissolved in dry benzene ($50cm^3$) was added dropwise to a cooled solution of phosphorus tribromide (1.5g) in 5:1 benzene-pyridine ($24cm^3$). The mixture was stirred at 0° for 30 mins., followed by removal of the volatile material to give a yellow oil which was taken up in ether ($25cm^3$), washed with 2M hydrochloric acid ($3 \times 15cm^3$) and dried ($MgSO_4$). Evaporation of the ether left 1.0g of a yellow oil which could not be made to crystallise and was identified as α-methyl-3,4,5-trimethoxystyrene[376] 323. $\tau(CDCl_3)$: 3.33 (2H, s, aromatics); 4.73, 4.95 (2H, 2s, vinylics); 6.12, 6.14 (9H, 2s, $3CH_3O$); 7.87 (3H, s, CH_3).

(b) The tertiary alcohol 319 (0.5g, 2mmol) was dissolved in dry

tetrahydrofuran ($10cm^3$) containing conc. hydrochloric acid ($1cm^3$). The solution was left standing for 24 hr. after which time the tetrahydrofuran was washed with 1M sodium carbonate solution ($3 \times 25cm^3$) and dried over $MgSO_4$. The volume was reduced to $2cm^3$ and passed down a silica column (15g) using chloroform as eluant, to give the ∝-methyl styrene 323 (0.45g, 98%).

(c) An equimolar mixture of the alcohol 319 (0.5g, 2mmol) and triphenylphosphine (0.58g)in carbon tetrachloride ($5cm^3$) was heated on a steam bath for 1 hr. and after column chromatography afforded a quantitative yield of 323.

(d) Methyl sulphide ($0.35cm^3$) was added dropwise to a stirred solution of N-chlorosuccinimide[344] (0.58g) in anhydrous dichloromethane ($20cm^3$) at 0°. The mixture was cooled to between -20° and -25° followed by introduction of alcohol 319(0.90g, 4mmol) dissolved in dichloromethane ($2cm^3$). Stirring was continued for 3 hr. at 0° after which the solution was poured into ice-cold brine ($20cm^3$) and extracted with ether ($2 \times 8cm^3$). The organic phase was washed with brine ($3 \times 20cm^3$), dried ($MgSO_4$) and evaporated to dryness giving 0.76g of a pale yellow oil identified as styrene 323.

∝,∝-Dimethyl-3,4,5-trimethoxybenzylmethylsulphonate 324

To a solution of alcohol 319 (1.0g, 4mmol) in dry pyridine cooled to 10° was added methanesulphonyl chloride (0.6g, 5mmol) dropwise, with vigorous stirring. Standing for 12 hr. followed by pouring onto crushed ice (25g) failed to give the expected solid. The aqueous phase was extracted with chloroform ($3 \times 20cm^3$) which in turn was washed with 2M hydrochloric acid ($3 \times 25cm^3$), dried ($MgSO_4$) and the chloroform removed

under reduced pressure to give 0.3g of a brown oil. Trituration with ether-petrol (b.p. 40-60°) produced 0.25g (19%) of α,α-dimethyl-3,4,5-trimethoxybenzylmethylsulphonate 324, m.p. 145-6°, as a colourless solid. $\tau(CDCl_3)$: 3.15 (2H, s, aromatics); 5.88 (3H, s, CH_3-SO_2); 6.12 (9H, s, $3CH_3O$); 8.08 (6H, s, $2CH_3$). Attempts to duplicate the above experiment were unsuccessful giving instead the elimination product α-methyl-3,4,5-trimethoxystyrene 323 in almost quantitative amounts. Alternative solvents, collidine or chloroform with triethylamine also produced only elimination product 323.

5.3 Anomalous Product from the Reaction of Amino-Acid 166 and Trifluoroacetic Anhydride in Pyridine

A refluxing mixture of amino-acid 166 (3.0g, 7.4mmol) and trifluoroacetic acid (11.0g, 0.53mmol) in dry pyridine (150cm^3) gave almost immediately upon boiling an intensely yellow coloured solution. Refluxing was continued for a further 30 mins, followed by pouring into chloroform (300cm^3), washing with 2M hydrochloric acid (5 x 200cm^3), water (3 x 150cm^3) and drying of the solvent over $MgSO_4$. The solvent was removed under reduced pressure, leaving a dark brown residue, which afforded after trituration with ether 3.0g of an homogenous yellow crystalline product, m.p. 197-8° (meths). (Found: C, 53.9; H, 3.9; N, 2.4; F, 21.7. $C_{24}H_{21}F_6NO_6$ requires C, 54.0; H, 3.9; N, 2.6; F, 21.4%). Found m/e = 533.1275, 464.1187 (100%). (Calc for $C_{24}H_{21}F_6NO_6$ 533.1274 and for $C_{23}H_{21}F_3NO_6$ 464.1321). ν(CDCl$_3$): 3200cm^{-1} (O-H); 1595cm^{-1} (COCF$_3$); 1550cm^{-1} (C=C). τ(CDCl$_3$): 2.01, 3.00, 3.13 (3H, 3s, aromatics); 3.72 (1H, broad s, exchanges with D_2O, O<u>H</u>), 4.50 (1H, q, J = 7.5Hz, C<u>H</u>CF$_3$); 5.98, 6.02, 6.12, 6.14 (12H, 4s, 4C<u>H</u>$_3$O).

^{19}F n.m.r. showed a singlet at 70.74 ppm (3F, COC<u>F</u>$_3$) and a doublet at 75.47 ppm (3F, J = 7.1Hz, CHC<u>F</u>$_3$), both signals being observed upfield of the $CFCl_3$ standard signal.

UV (ethanol); neutral and pH1: 226, 259, 295, 322 and 430nm; pH 14: 229, 278, 304, 376 and 453nm. Based upon the above information the unknown compound was identified as <u>2,9,10,11-tetramethoxy-3-hydroxy-8-trifluoromethyl-13-trifluoroacetyl-7,8-dihydroprotoberberine 326</u>

The phenolic compound (0.2g, 3.7mmol) was dissolved in ether (50cm^3) and an ethereal solution of diazomethane added with stirring. After standing at room temperature for 3 hr. excess diazomethane was destroyed by the addition of glacial acetic acid (2cm^3). The ethereal layer was washed with saturated sodium bicarbonate solution (3 x 50cm^3), water (3 x 50cm^3) and dried ($MgSO_4$). Evaporation of the solvent gave yellow platelets

(aqueous meths) of 2,3,9,10,11-pentamethoxy-8-trifluoromethyl-13-trifluoroacetyl-7,8-dihydroprotoberberine 327, m.p. 205-6°. (Found: C, 54.6; H, 4.4; N, 2.7; F, 21.6. $C_{25}H_{23}F_6NO_6$ requires C, 54.8; H, 4.2; N, 2.6; F, 20.8%). Found m/e = 547.1388, 478.1497 (100%). (Calc for $C_{25}H_{23}F_6NO_6$ 547.1430 and for $C_{24}H_{23}F_3NO_6$ 478.1478). $\nu(CDCl_3)$: 1633cm^{-1} ($COCF_3$); 1585cm^{-1} (C=C). $\tau(CDCl_3)$: 2.02, 2.95, 3.20 (3H, 3s, aromatics); 4.48 (1H, q, J = 7.5Hz, $C\underline{H}CF_3$); 5.98, 6.02, 6.04, 6.13, 6.15 (15H, 5s, $5C\underline{H}_3O$). ^{19}F nmr showed a doublet centred at 75.47ppm (J = 7.5Hz) and a singlet at 70.74ppm. UV (ethanol) showed absorption maxima at 225, 255, 293, 322 and 430nm, no change being observed in either acidic or basic media.

5.4 Enzymic Oxidative Studies on Corypalline 328

Corypalline 328 (0.5g, 2.6mmol) was dissolved in 0.05M sodium methoxide in methanol ($50cm^3$). The methanol was removed to leave the corresponding sodium salt which was dissolved in water ($10cm^3$) and added to a solution of tetraethylammonium perchlorate (3.5g) in acetonitrile ($150cm^3$). This solution was oxidised anodically according to the method of Bobbitt,[352] at 0.0mV with respect to the standard calomel electrode. The electrolysis was stopped after 65 mins. due to coating of the electrodes. The reaction mixture was filtered, made acidic (2M hydrochloric acid), then basic (ammonia solution) and evaporated to dryness, followed by trituration of the residue with ethanol, to give the dimeric product 329 (0.11g, 22%), m.p. 228-34° (lit 235-7°).[352] τ(DMSO): 3.50 (2H, s, aromatics); 6.24 (6H, s, $2CH_3O$); 8.00 (6H, s, $2NCH_3$).

Enzymic oxidation of corypalline (0.1g) using a crude laccase preparation was carried out in a solution of phosphate buffer (pH6, $10cm^3$) containing ethanol ($2cm^3$). The laccase solution ($2.5cm^3$) was added every 30 mins. over 3 hr. and the mixture left for 21 hr., followed by extraction with chloroform (3 x $10cm^3$) and drying ($MgSO_4$). T.l.c. (methanol-ammonia, 97:3, silica gel) showed the presence of dimeric compound 329 as well as a substantial amount of unreacted starting material.

A similar oxidation using horseradish peroxidase solution (50mg in $50cm^3$ water, $3cm^3$) and hydrogen peroxide (0.87mM, $3cm^3$) also exhibited the presence of 329 by t.l.c. also after 21 hr. A corypalline blank solution, when allowed to stand for the same period of time under the same conditions showed only unreacted corypalline.

BIBLIOGRAPHY

1. J.M. Bobbitt and T.Y. Cheng, J. Org. Chem., 1976, 41, 443.

2. G. Hahn and F. Rumpf, Chem. Ber., 1938, 71, 2141.

3. P.D. McDonald and G.A. Hamilton, in "Oxidation in Organic Chemistry" (W.S. Trahanovsky, Ed.), Academic Press, New York, 1973, Chap. 2.

4. D.H.R. Barton and T. Cohen, "Festschrift A. Stoll", Birkhäuser, Basel, 1957, p. 117.

5. H. Erdtman and C.A. Wachtmeister, "Festschrift A. Stoll", Birkhäuser, Basel, 1957, p. 144.

6. B.S. Thyagarajan, Chem. Rev., 1958, 58, 439.

7. C.H. Hassall and A.I. Scott, in "Recent Developments in the Chemistry of Natural Phenolic Compounds", (W.D. Ollis, Ed.), Pergammon, Oxford, 1961.

8. J.R. Lewis, Chem. and Ind., 1962, 159; 1964, 1672.

9. A.R. Battersby, Proc. Chem. Soc., 1963, 189.

10. D.H.R. Barton, Proc. Chem. Soc., 1963, 293.

11. H. Musso, Angew. Chem. Int. Ed., 1963, 2, 723.

12. A.I. Scott, Quart. Rev., 1965, 19, 1.

13. H. Musso (Chap.1) and A.I. Scott (Chap.2), in "Oxidative Coupling of Phenols", (W.I. Taylor and A.R. Battersby, Eds.), Arnold, London, 1968.

14. M.L. Mikailovic and Z. Cekovic, in "The Chemistry of the Hydroxyl Group", (S. Patai, Ed.), J. Wiley and Sons, London, 1970, Chap. 10.

15. J.M. Gulland and R. Robinson, Mem. Proc. Manchester Lit. Phil. Soc., 1925, 69, 79.

16. R. Pummerer, H. Puttfarcken and P. Schopflocher, Chem. Ber., 1925, 58, 1808.

17. D.H.R. Barton, Chem. Brit., 1967, 3, 330.

18. A. Rieker, N. Zeller, K. Schur and E. Muller, Annalen, 1966, 697, 1.

19. G. Williams, Chem. Soc., Spec. Publ., 1970, 24, 25.

20. W.A. Waters, J. Chem. Soc. (B), 1971, 2026.

21. R.A. Anderson, D.T. Dalgleish, D.C. Nonhebel and P.L. Pauson, J. Chem. Research (S), 1977, 12.

22. T.J. Stone and W.A. Waters, J. Chem. Soc., 1964, 213.

23. T.J. Stone and W.A. Waters, J. Chem. Soc., 1964, 4302.

24. C.D. Cook, C.B. Depatie and E.S. English, J. Org. Chem., 1959, 24, 1356.

25. C.D. Cook, J. Org. Chem., 1953, 18, 261.

26. A.R. Forrester, J.M. Hay and R.H. Thomson, in "Organic Chemistry of Stable Free Radicals", Academic Press, New York, 1968, Chap. 7.

27. E.J. Land and G. Porter, Trans. Faraday Soc., 1963, 59, 2016.

28. G. Dobson and L.I. Grossweiner, Trans. Faraday Soc., 1965, 61, 708.

29. R. Stebbins and F. Sicilio, Tetrahedron, 1970, 26, 291.

30. C.D. Cook, N.G. Nash, and H.R. Flanagan, J. Amer. Chem. Soc., 1955, 77, 1783.

31. C.D. Cook and B.E. Norcross, J. Amer. Chem. Soc., 1956, 78, 3797, ibid, 1959, 81, 1176.

32. C.G. Haynes, A.H. Turner and W.A. Waters, J. Chem. Soc., 1956, 2823.

33. R. Cecil and J.S. Littler, J. Chem. Soc. (B), 1968, 1420.

34. P.D. McDonald and G.A. Hamilton, J. Amer. Chem. Soc., 1973, 95, 7752.

35. F.J. Vermillian, Jnr. and I.A. Pearl, J. Electrochem. Soc., 1964, 111, 1392.

36. C.A. Chambers and J.Q. Chambers, J. Amer. Chem. Soc., 1966, 88, 2922.

37. F.W. Steuber and K. Dimroth, Chem. Ber., 1966, 99, 258.

38. A.B. Suttie, Tetrahedron Lett., 1969, 953.

39. A. Ronlán and V.D. Parker, J. Chem. Soc. (C), 1971, 3214.

40. U. Palmquist, A. Ronlán and V.D. Parker, Acta Chem. Scand. Ser B, 1974, 28, 267.

41. U. Palmquist, A. Nilsson, V.D. Parker and A. Ronlán, J. Amer. Chem. Soc., 1976, 98, 2571.

42. A. Nilsson, A. Ronlán and V.D. Parker, J. Chem. Soc. Perkin Trans I, 1973, 2337.

43. U. Svanholm, K. Bechgaard and V.D. Parker, J. Amer. Chem. Soc., 1974, 96, 2409.

44. M. Chauhan, F.M. Dean, K. Hindley and M. Robinson, Chem. Comm., 1971, 1141.

45. D.G. Hewitt, J. Chem. Soc.(C), 1971, 1750.

46. K. Dimroth, W. Umbach, and H. Thomas, Chem. Ber, 1967, 100, 132.

47. G. Popp and N.C. Reitz, J. Org. Chem., 1972, 37, 3646.

48. J.C. Evans and P.J. Emes, in "MTP International Review of Science-Free Radical Reactions", (W.A. Waters, Ed.), Butterworths, London, 1973, Chap. 10.

49. J.Q. Chambers, in "MTP International Review of Science-Free Radical Reactions", (W.A. Waters, Ed.), Butterworths, London, 1975, Chap. 10.

50. V.D. Parker and A. Ronlán, J. Amer. Chem. Soc., 1975, 97, 4714.

51. J.R. Falck, L.L. Miller and F.R. Stermitz, J. Amer. Chem. Soc., 1974, 96, 2981.
L.L. Miller, F.R. Stermitz, J.Y. Becker and V. Ramachandran, J. Amer. Chem. Soc., 1975, 97, 2922.
J.Y. Becker, L.L. Miller and F.R. Stermitz, J. Electroanal. Chem., 1976, 68, 181.

52. M. Sainsbury and J. Wyatt, J. Chem. Soc. Perkin Trans I, 1977, 1750.

53. E. Kotani, N. Takeuchi and S. Tobinaga, Chem. Comm., 1973, 550.

54. J.S. Davies, C.H. Hassall and J.A. Schofield, J. Chem. Soc., 1964, 3126.

55. A.I. Scott, P.A. Dodson, F. McCapra and M.B. Myers, J. Amer. Chem. Soc., 1963, 85, 3702.

56. R. Criegee, in "Oxidation in Organic Chemistry", (K.B. Wiberg, Ed.), Part A, Academic Press, New York, 1965, Chap. 5.

57. M.J. Harrison and R.O.C. Norman, J. Chem. Soc.(C), 1970, 728.

58. H. Funk, W. Weiss and M. Zeisung, Z. Anorg. Allg. Chem., 1958, 36, 296.

59. W.L. Carrick, G.L. Karapinka and G.T. Kwiatkowski, J. Org. Chem., 1969, 34, 2388.

60. M.A. Schwartz, B.F. Rose, R.A. Holton, S.W. Scott and B. Vishnuvajjala, J. Amer. Chem. Soc., 1977, 99, 2571.

61. M.A. Schwartz, B.F. Rose and B. Vishnuvajjala, J. Amer. Chem. Soc., 1973, 95, 612.

62. A. McKillop, D.H. Perry, M. Edwards, S. Antus, L. Farkas, M. Nógrádi and E.C. Taylor, J. Org. Chem., 1976, 41, 282.

63. J.W.A. Findlay, P. Gupta and J.R. Lewis, J. Chem. Soc. (C), 1969, 2761.

64. J.W.A. Findlay and A.B. Turner, J. Chem. Soc. (C), 1971, 23.

65. (a) M. Cornia and L. Merlini, Chem. Comm., 1975, 428.

(b) I.M. Campbell, C.H. Calzadilla and N.J. McCorkindale, Tetrahedron Lett., 1966, 5107.

66. R.A. Abramovitch, G. Alvernhe and M.N. Inbasekaran, Tetrahedron Lett., 1977, 1113.

67. R. Robinson, J. Chem. Soc., 1917, 876.

68. C. Schöpf and H. Bayerle, Annalen, 1934, 513, 190.

69. C. Schöpf and W. Salzer, Annalen, 1940, 544, 1.

70. D.S. Bhakuni, J. Sci. Ind. Res., 1976, 35, 461.

71. A.R. Battersby, in "Oxidative Coupling of Phenols", (W.I. Taylor and A.R. Battersby, Eds.), Arnold, London, 1967, Chap. 3.

72. M. Shamma and W.A. Slusarchyk, Chem. Rev., 1964, 64, 59.

73. B. Miller, in "Mechanisms of Molecular Migrations", (B.S. Thyagarajan, Ed.), Interscience 1968.

74. R. Baird and S. Winstein, J. Amer. Chem. Soc., 1962, 84, 788.

75. B. Gilbert, M.E.A. Gilbert, M.M. de Oliveira, O. Ribeiro, E. Wenkert, B. Wickberg, U. Hollstein and H. Rapoport, J. Amer. Chem. Soc., 1964, 86, 694.

76. R.T. Arnold and J.S. Buckley, J. Amer. Chem. Soc., 1949, 71, 1781.

77. D.H.R. Barton, A.M. Deflorin and O.E. Edwards, J. Chem. Soc., 1956, 530.

78. V. Arkley, F.M. Dean, A. Robertson and P. Sidisunthorn, J. Chem. Soc., 1956, 2322.

79. G. Blaschke, Arch. Pharm., 1968, 301, 432.

80. G. Blaschke, Arch. Pharm., 1970, 303, 358.

81. G. Blaschke, G. Waldheim, M. von Schantz and P. Peura, Arch. Pharm., 1974, 307, 122.

82. S. Tewari, D.S. Bhakuni and R.S. Kapil, Chem. Comm., 1974, 940.

83. E. Brochmann-Hanssen, C.-C. Fu and L.Y. Misconi, J. Pharm. Sci., 1971, 60, 1880.

84. E. Brochmann-Hanssen, C.-H. Chen, H.-C. Chiang, C.-C. Fu and H. Nemoto, J. Pharm. Sci., 1973, 62, 1291.

85. E. Brochmann-Hanssen, C.-H. Chen, H.-C. Chiang and K. McMurtrey, Chem. Comm., 1972, 1269.

86. D.H.R. Barton, D.S. Bhakuni, G.M. Chapman, and G.W. Kirby, J. Chem. Soc., 1967, 2134.

87. D.H.R. Barton, D.S. Bhakuni, G.M. Chapman and G.W. Kirby, J. Chem. Soc., 1966, 250.

88. D.H.R. Barton, R.H. Hesse and G.W. Kirby, J. Chem. Soc., 1965, 6379.

89. A.R. Battersby, T.J. Brocksom and R. Ramage, Chem. Comm., 1969, 464.

90. A.R. Battersby, R.T. Brown, J.H. Clements and G.G. Iverach, Chem. Comm., 1969, 230.

91. D.S. Bhakuni, S. Satish, H. Uprety and R.S. Kapil, Phytochemistry, 1974, 13, 2767.

92. L.J. Haynes, K.L. Stuart, D.H.R. Barton, D.S. Bhakuni and G.W. Kirby, Chem. Comm., 1965, 141.

93. D.H.R. Barton, D.S. Bhakuni, G.M. Chapman, G.W. Kirby, L.J. Haynes and K.L. Stuart, J. Chem. Soc., 1967, 1295.

94. A.R. Battersby, J.L. McHugh, J. Staunton and M. Todd, Chem. Comm., 1971, 985.

95. A.R. Battersby, P. Böhler, M.H.G. Munro and R. Ramage, Chem. Comm., 1969, 1066. J. Chem. Soc. Perkin I, 1974, 1399.

96. A.R. Battersby, E. McDonald, M.H.G. Munro and R. Ramage, Chem. Comm., 1967, 934.

97. A.R. Battersby, R.B. Herbert, E. McDonald, R. Ramage and J.H. Clements, J. Chem. Soc. Perkin I, 1972, 1741.

98. A.R. Battersby, P.W. Sheldrake and J.A. Milner, Tetrahedron Lett., 1974, 3315.

99. N.J. McCorkindale, D.S. Magrill, M. Martin-Smith, S.J. Smith and J.B. Stenlake, Tetrahedron Lett., 1964, 3841.

100. (a) R.H.F. Manske, J. Amer. Chem. Soc., 1950, 72, 55.
(b) M.F. Grundon, Prog. Org. Chem., 1964, 6, 38.
(c) J.McShefferty, P.F. Nelson, J.L. Paterson, J.B. Stenlake and J.P. Todd, J. Pharm. Pharmacol., 1956, 8, 117.

101. T. Kametani, K. Fukumoto and M. Fujihara, Chem. Comm., 1971, 352.

102. T. Kametani, T. Kikuchi and K. Fukumoto, Chem. Comm., 1967, 546, Chem. Pharm. Bull., 1968, 16, 1003.

103. A.R. Battersby, R. Binks, D.M. Foulkes, R.J. Francis, D.J. McCaldin and H. Ramuz, Proc. Chem. Soc., 1963, 203.

104. A.R. Battersby, D.M. Foulkes and R. Binks, J. Chem. Soc., 1965, 3323.

105. D.H.R. Barton, G.W. Kirby, W. Steglich and G.M. Thomas, Proc. Chem. Soc., 1963, 203.

106. D.H.R. Barton, G.W. Kirby, W. Steglich, G.M. Thomas, A.R. Battersby, T.A. Dobson and H. Ramuz, J. Chem. Soc., 1965, 2423.

107. J. Kalvoda, P. Buchschacher and O. Jeger, Helv. Chim. Acta, 1955, 38, 1847.

108. G. Kartha, F.R. Ahmed and W.H. Barnes, Acta Cryst., 1962, 15, 326.

109. E. Brochmann-Hanssen and B. Nielsen, Tetrahedron Lett., 1965, 1271.

110. A.R. Battersby, G.W. Evans, R.O. Martin, M.E. Warren and H. Rapoport, Tetrahedron Lett., 1965, 1275.

111. K.L. Stuart, V. Teetz and B. Franck, Chem. Comm., 1969, 333.

112. K.L. Stuart and L. Graham, Phytochemistry, 1973, 12, 1967.

113. D.H.R. Barton, A.J. Kirby and G.W. Kirby, Chem. Comm., 1965, 52.

114. D.H.R. Barton, A.J. Kirby and G.W. Kirby, J. Chem. Soc. (C), 1968, 929.

115. R. Robinson and S. Sugasawa, J. Chem. Soc., 1932, 789. ibid, 1933, 280.

116. A.R. Battersby, R.C.F. Jones, R. Kazlauskas, C. Poupat, C.W. Thomber, S. Ruchirawat and J. Staunton, Chem. Comm., 1974, 773.

117. A.R. Battersby, A. Minta, A.P. Ottridge and J. Staunton, Tetrahedron Lett., 1977, 1321.

118. D.H.R. Barton, R.B. Boar and D.A. Widdowson, J. Chem. Soc.(C), 1970, 1213.

119. Y. Inubushi, H. Furukawa and M. Ju-ichi, Tetrahedron Lett., 1969, 153.

120. D.H.R. Barton, C.J. Potter and D.A. Widdowson, J. Chem. Soc. Perkin I, 1974, 346.

121. D.H.R. Barton, R.D. Bracho, C.J. Potter and D.A. Widdowson, J. Chem. Soc. Perkin I, 1974, 2278.

122. M. Shamma, in "The Isoquinoline Alkaloids", Academic Press, New York, 1972.

123. A.R. Battersby, R. Binks and R. Huxtable, Tetrahedron Lett., 1967, 563.

124. A. Pictet and T. Spengler, Chem. Ber., 1911, 44, 2030.

125. E. Spath, Monatsh., 1921, 42, 97.

126. E. Winterstein and G. Trier, in "Die Alkaloide", Borntraeger, Bonn, 1910, p. 307.

127. A.R. Battersby, A.R. Burnett and P.G. Parsons, J. Chem. Soc. (C), 1969, 1187.

128. A.R. Battersby and B.J.T. Harper, J. Chem. Soc., 1962, 3526.

129. I.D. Spenser and J.R. Gear, J. Amer. Chem. Soc., 1962, 84, 1059.

130. B.S. Bhakuni, A.N. Singh, S. Tewari and R.S. Kapil, J. Chem. Soc. Perkin I, 1977, 1662.

131. E. Leete and J.D. Braunstein, Tetrahedron Lett., 1969, 451.

132. M. Slaytor and I.J. McFarlane, Phytochemistry, 1968, 7, 605.

133. G.J. Kapadia, G.S. Rao, E. Leete, M.B.E. Fayez, Y.N. Vaishnav and H.M. Fales, J. Amer. Chem. Soc., 1970, 92, 6943.

134. J. Lundstrom, Acta Pharm. Suecica, 1971, 8, 485.

135. I.J. McFarlane and M. Slaytor, Phytochemistry, 1972, 11, 229.

136. W.H. Whalley and T.R. Govindachari, Org. Reactions, 1951, 6, 151.

137. K. Stolle and D. Gröger, Arch. Pharm., 1968, 301, 561.

138. G.J. Kapadia, M.B.E. Fayez, Y.S. Vaishnav, H.M. Fales and G.S. Rao, Lloydia, 1969, 32, 525.

139. I.J. McFarlane and M. Slaytor, Phytochemistry, 1972,11, 235.

140. M.L. Wilson and C.J. Coscia, J. Amer. Chem. Soc., 1975, 97, 431.

141. A.R. Battersby, R.C.F. Jones and R. Kazlauskas, Tetrahedron Lett., 1975, 1873.

142. S. Doonan, in "The Chemistry of Carboxylic Acids and Esters" (S. Patai, Ed.), J. Wiley and Sons, London, 1969, Chap. 18.

143. E.A. Boeker and E.E. Snell, in "The Enzymes", (P.D. Boyer, Ed.), 3rd Edition, Vol VI, Academic Press, New York, 1972, Chap. 7.

144. T.A. Geissman and D.H.G. Crout, in "Organic Chemistry of Secondary Plant Metabolism", Freeman Cooper and Co., San Francisco, 1969, p. 435.

145. O. Schales, in "The Enzymes", (J.B. Sumner and K. Myrbäck, Eds.), 1st Edition, Vol III, Academic Press, New York, 1951, 216.

146. J. Baddiley and E.F. Gale, Nature, 1945, 155, 727.

147. W.D. Bellamy and I.C. Gunsalus, J. Bacteriol., 1945, 50, 95.

148. D. Heyl, E. Luz, S.A. Harris and K. Folkers, J. Amer. Chem. Soc., 1951, 73, 3436.

149. J. Baddiley and A.P Mathias, J. Chem. Soc., 1952, 2583.

150. D.E. Metzler, M. Ikawa and E.E. Snell, J. Amer. Chem. Soc., 1954, 76, 648.

151. G.D. Kalyankar and E.E. Snell, Biochemistry, 1962, 1, 594.

152. H. Kolbé, Ann. Chem., 1849, 69, 257.

153. B.C.L. Weedon, Adv. in Org. Chem., 1960, 1, 1.

154. L. Eberson, in "The Chemistry of Carboxylic Acids and Esters" (S. Patai, Ed.), J. Wiley and Sons, London, 1969, Chap. 2.

155. L. Eberson, in "Organic Electrochemistry", (M.M. Baizer, Ed.), Marcel Dekker Inc., New York, 1973, Chap. 13.

156. L. Eberson, Acta Chem. Scand., 1963, 17, 2004.

157. Y. Takayama, Bull. Chem. Soc. Japan, 1933, 8, 178.

158. P.G. Gassman and B.L. Fox, J. Org. Chem., 1967, 32, 480.

159. R.P. Linstead, B.R. Shephard and B.C.L. Weedon, J. Chem. Soc., 1951, 2854.

160. J.P. Coleman, J.H.P. Utley and B.C.L. Weedon, Chem. Comm., 1971, 438.

161. J.P. Coleman and L. Eberson, Chem. Comm., 1971, 1300.

162. J.M. Bobbitt, C. Kulkarni and P Wiriyachitra, Heterocycles, in press.

163. M.L. Wilson and C.J. Coscia, manuscript in preparation.

164. V. Ullrich and W. Duppel, in "The Enzymes", (P.D. Boyer, Ed.), 3rd Edition, Vol XII, Academic Press, New York, 1975, p. 296.

165. B.G. Malmstrom, L.-E. Andréasson and B. Reinhammer, in "The Enzymes", (P.D. Boyer, Ed.), 3rd Edition, Vol XII, Academic Press, New York, 1975, p. 557.

166. D.R. McMillin, R.C. Rosenberg and H.B. Gray, Proc. Nat. Acad. Sci., U.S.A., 1974, 71, 4760.

167. V. Miskowski, S.-P. Tang, T.G. Spiro, E. Shapiro and T.H. Moss, Biochemistry, 1975, 14, 1244.

168. E.I. Solomon, J. Rawlings, D.R. McMillin, P.J. Stephens and H.B. Gray, J. Amer. Chem. Soc., 1976, 98, 8046.

169. O. Siiman, N.M. Young and P.R. Carey, J. Amer. Chem. Soc., 1976, 98, 744.

170. G. Bertrand, C.R. Acad. Sci., 1894, 118, 1215.

171. R. Blaich and K. Esser, Arch. Microbiol., 1975, 103, 271.

172. J. Boidin, Revue de Mycologie, 1951, 16, 173.

173. W.M. Dion, Can. J. Bot., 1952, 30, 9.

174. L. Schanel and K. Esser, Arch. Microbiol., 1971, 77, 111.

175. G. Fahreus and B. Reinhammer, Acta Chem. Scand., 1967, 21, 2367.

176. H.A. Neufeld, F.M. Latterell, L.F. Green and R.L. Weintraub, Arch. Biochem. Biophys., 1958, 76, 317.

177. W.F. van Vliet, Biochem. et Biophys. Acta, 1954, 15, 211.

178. K. Law, Ann. Bot., 1950, 14, 69.

179. M. Grauberd, Enzymologia, 1938, 5, 332.

180. K. Law, Ann. Bot., 1955, 19, 561.

181. G. Fahreus, Ann. Royal Agric. Coll. Sweden, 1949, 16, 618.

182. B.G. Malmström, B. Reinhammer and T. Vanngard, Biochem. et Biophys. Acta, 1968, 156, 67.

183. R. Malkin, B.G. Malmström and T. Vanngard, Eur. J. Biochem., 1969, 7, 253.

184. L.-E. Andréasson and T. Vanngard, Biochem. et Biophys. Acta, 1970, 200, 247.

185. F.X.R. van Leevwen, R. Wever and B.F. van Gelder, Biochem. et Biophys. Acta, 1973, 315, 200.

186. R. Malkin, B.G. Malmström and T. Vanngard, Eur. J. Biochem., 1969, 10, 324.

187. B. Reinhammer, Biochem. et Biophys. Acta, 1972, 275, 245.

188. L. Broman, B.G. Malmström, R. Aasa and T. Vanngard, Biochem. et Biophys. Acta, 1963, 75, 365.

189. H.S. Mason, E. Spencer and I. Yamazaki, Biochem. Biophys. Res. Comm., 1961, 4, 236.

190. B.G. Malmström, L.-E. Andréasson and B. Reinhammer, in "The Enzymes" (P.D. Boyer, Ed.), 3rd Edition, Vol XII, Academic Press, New York, 1975, p. 575.

191. G.A. Hamilton, Adv. Enzymology, 1969, 32, 55.

192. R. Malkin and B.G. Malmström, Adv. Enzymology, 1970, 33, 177.

193. G. Benfield, S.M. Bocks, K. Bromley and B.R. Brown, Phytochemistry, 1964, 3, 79.

194. S.M. Bocks, R.C. Cambie and T. Takahashi, Tetrahedron, 1963, 19, 1109.

195. For reviews see:-
(a) A.S. Brill, in "Comprehensive Biochemistry", (M. Florkin and E.H. Stotz, Eds.), Elsevier, Amsterdam, 1966, Vol.14, p. 447.
(b) B.C. Saunders, A.G. Holmes-Siedle and B.P. Stark, in "Peroxidase", Butterworths, London, 1964.

196. D. Keilin and T. Mann, Proc. Royal Soc. B, 1937, 122, 119.

197. H. Theorell, Arkiv. Kemi. Mineral Geol., 1942, 15B, No. 24.

198. P. George, Biochem. J., 1953, 54, 267.

199. I. Yamazaki and K. Yokota, Biochem. et Biophys. Acta, 1965, 105, 301.

200. G.R. Schonbaum and S. Lo, J. Biol. Chem., 1972, 247, 3353.

201. A.S. Brill and H.E. Sandberg, Biochemistry, 1968, 7, 4254.

202. V. Katovic, L.T. Taylor and D.H. Busch, J. Amer. Chem. Soc, 1969, 91, 2122.

203. D. Dolphin, R.H. Felton, D.C. Borg, and J. Fajer, J. Amer. Chem. Soc., 1970, 92, 743.

204. I. Yamazaki, H.S. Mason and L. Piette, J. Biol. Chem., 1960, 235, 2444.

205. B. Chance, Science, 1949, 109, 204.

206. J.E. Critchlow and H.B. Dunford, J. Biol. Chem., 1972, 247, 3703. ibid, 1972, 247, 3714.

207. E.E. Conn, in "Biochemistry of Phenolic Compounds", (J.B. Harborne, Ed.), Academic Press, New York, 1964, p. 429.

208. M.A. Joslyn, Adv. Enzymology, 1949, 9, 613.

209. K. Freudenberg, Nature, 1959, 183, 1152.

210. H. Mason, L. Onopryenko and D.H. Buchler, Biochem. et Biophys. Acta, 1957, 24, 225.

211. M. Mazelis, J. Biol. Chem., 1962, 237, 104.

212. K.H. Frömming, Arch. Pharm., 1967, 300, 977.

213. T. Kametani, S. Takano and T. Kobari, Tetrahedron Lett., 1968, 4565; J. Chem. Soc.(C), 1969, 9.

214. T. Kametani, S. Takano and T. Kobari, J. Chem. Soc.(C), 1969, 2770.

215. T. Kametani, H. Nemoto, T. Kobari and S. Takano, J. Heterocyclic Chem., 1970, 7, 181.

216. T. Kametani, S. Takano and T. Kobari, J. Chem. Soc.(C), 1971, 1030.

217. T. Kametani, K. Fukumoto, K. Kigasawa and K. Wakisaka, Chem. Pharm. Bull., 1971, 19, 714.

218. Y. Inubushi, Y. Aoyagi and M. Matsuo, Tetrahedron Lett., 1969, 2363.

219. J.M. Bobbitt and R.C. Hallcher, Chem. Comm., 1971, 543.

220. K.L. Stuart and A. Callender, Rev. Latinamer. Quim., 1972, 3, 19.

221. A. Brossi, A. Ramel, J. O'Brien and S. Teitel, Chem. Pharm. Bull., 1973, 21, 1839.

222. B. Franck, G. Blaschke and G. Schlingloff, Angew. Chem. Int. Ed., 1964, 3, 192.

223. M.A. Pickard and D.W.S. Westlake, Can. J. Microbiol., 1969, 15, 869.

224. T.M. Devlin, in "Methods in Enzymology", (S.P. Colowick and N.O. Kaplan, Eds.), Academic Press, New York, 1955, Vol. 2, 770.

225. J.M. Bobbitt, A.S. Steinfeld, K.H. Weisgraber and S Dutta, J. Org. Chem., 1969, 34, 2478.

226. J. Finkelstein, J. Amer. Chem. Soc., 1951, 73, 550.

227. S. Teitel and A. Brossi, J. Heterocyclic Chem., 1968, 5, 825.

228. W.H. Whalley and T.R. Govindachari, Org. Reactions, 1951, 6, 74.

229. D.G. Harvey, E.J. Miller and W. Robson, J. Chem. Soc., 1941, 153.

230. S. Agurell, Lloydia, 1969, 32, 40.

231. F. Mauthner, Chem. Ber., 1908, 41, 3662.

232. W. Baker and R. Robinson, J. Chem. Soc., 1929, 152.

233. H.R. Snyder, J.S. Buck and W.S. Ide, Org. Syn. Coll., Vol. 2, p. 333.

234. M. Shamma and C.D. Jones, J. Org. Chem., 1970, 35, 3119.

235. F.D. Popp, Adv. in Heterocyclic Chem., 1968, 9, 1.

236. M.J. Bevis, E.J Forbes, N.N. Naik and B.C. Uff, Tetrahedron, 1971, 27, 1253.

237. W.E. McEwen and R.L. Cobb, Chem. Rev., 1955, 55, 511.

238. D.J. Byron, G.W. Gray and R.C. Wilson, J. Chem. Soc., 1966, 840.

239. J.M. Bobbitt and D.A. Scola, J. Org. Chem., 1960, 25, 560.

240. H.C. Brown and C.P. Garg, J. Amer. Chem. Soc., 1964, 86, 1085.

241. H. Bruderer and A. Brossi, Helv. Chim. Acta, 1965, 48, 1945.

242. M.R. Hamblin, Ph.D. Thesis, Trent Polytechnic, Nottingham, 1976.

243. G. Jonsson, Acta Chem. Scand., 1966, 20, 2755.

244. (a) M. Dixon and E.C. Webb, in "The Enzymes", Longmans, London, 1964, p. 64.
(b) ibid., p. 67.

245. H. Lineweaver and D. Burk, J. Amer. Chem. Soc., 1934, 56, 658.

246. T. Kametani, in "The Chemistry of the Isoquinoline Alkaloids", Elsevier Publishing Co., Amsterdam, 1969.

247. H. Guinaudeau, M. LeBoeuf and A. Cave, Lloydia, 1975, 38, 275.

248. M. Shamma and S.S. Salgar, in "The Alkaloids-Specialist Periodical Reports", 1974, Vol. 4, Chap. 9.

249. T. Kametani, Lect. Heterocyclic Chem., 1974, 2, 57.

250. T. Kametani, K. Fukumoto and F. Satoh, Bio-Org. Chem., 1974, 3, 430.

251. C.D. Hufford and J.M. Morgan, J. Org. Chem., 1976, 41, 375.

252. B.R. Pai and C.S. Swaminathan, Ind. J. Chem., 1973, 11, 1086.

253. J.L. Neumeyer, B.R. Neustadt and J.W. Weintraub, Tetrahedron Lett., 1967, 3107.

254. J.L. Neumeyer, F.E. Granchelli, K. Fuxe, U. Ungerstedt and H. Corrodi, J. Med. Chem., 1974, 17, 1090.

255. J.L. Neumeyer, W.P. Dafeldecker, B. Costall and R.J. Naylor, J. Med. Chem., 1977, 20, 190.

256. M.P. Cava, K.T. Buck, I. Noguchi, M. Srinivasan, M.G. Rao and A.I. DaRocha, Tetrahedron, 1975, 31, 1667.

257. W.S. Saari, S.W. King, V.J. Lotti and A. Scriabine, J. Med. Chem., 1974, 17, 1086.

258. F.D. Popp, Heterocycles, 1973, 1, 165.

259. D.F. DeTar, Org. Reactions, 1957, 9, 409.

260. D.R. Dalton and A.A. Abraham, Syn. Comm., 1972, 2, 303.

261. J.A. Weisbach and B. Douglas, J. Org. Chem., 1962, 27, 3738.

262. S.M. Kupchan, V. Kameswaran and J.W.A. Findlay, J. Org. Chem., 1973, 38, 405.

263. T. Kametani, T. Sugahara and K. Fukumoto, Chem. Pharm. Bull., 1974, 22, 966.

264. S.M. Kupchan and A.J. Liepa, J. Amer. Chem. Soc., 1973, 95, 4062.

265. T. Kametani, H. Sugi, S. Shibuya and K. Fukumoto, Chem. Pharm. Bull., 1971, 19, 1513.

266. S.V. Kessar, R. Randhawa, U.K. Nadir and S.S. Gandhi, Ind. J. Chem., 1975, 13, 1113.

267. T. Kametani, K. Fukumoto and T. Nakano, Tetrahedron, 1972, 28, 4667.

268. I. Ahmad and M.S. Gibson, Can. J. Chem., 1975, 53, 3660.

269. T. Kametani, A. Ujiie, K. Takahashi, T. Nakano, T. Suzuki and K. Fukumoto, Chem. Pharm. Bull., 1973, 21, 766.

270. S.V. Kessar, S. Batra, U.K. Nadir and S.S. Gandhi, Ind. J. Chem., 1975, 13, 1109.

271. T. Kametani, S. Shibuya and S. Kano, J. Chem. Soc. Perkin I, 1973, 1212.

272. S. Kano, Y. Takahagi, E. Komiyama, T. Yokomatsu and S. Shibuya, Heterocycles, 1976, 4, 1013.

273. M.A. Schwartz, Syn. Comm., 1973, 3, 33.

274. M.A. Schwartz and I.S. Mami, J. Amer. Chem. Soc., 1975, 97, 1239.

275. T. Kametani, Y. Satoh, M. Takemura, Y. Ohta, M. Ihara and K. Fukumoto, Heterocycles, 1976, 5, 173.

276. T. Kametani, M. Ihara, M. Takemura, Y. Satoh, H. Terasawa, Y. Ohta, K. Fukumoto and K. Takahashi, J. Amer. Chem. Soc., 1977, 99, 3805.

277. S.M. Kupchan and C.-K. Kim, J. Org. Chem., 1976, 41, 3210.

278. T. Kametani, K. Fukumoto and M. Fujihara, J. Chem. Soc. Perkin I, 1972, 394.

279. T. Kametani, K. Takahashi, K. Ogasawara and K. Fukumoto, Chem. Pharm. Bull., 1973, 21, 662.

280. S.M. Kupchan, C.-K. Kim and K. Miyano, Heterocycles, 1976, 4, 235.

281. T. Kametani and K. Fukumoto, Acc. Chem. Res., 1972, 5, 212.

282. M.P. Cava, P. Stern and K. Wakisaka, Tetrahedron, 1973, 29, 2245.

283. M.P. Cava and S.S. Libsch, J. Org. Chem., 1974, 39, 577.

284. S.M. Kupchan and P.F. O'Brien, Chem. Comm., 1973, 915.

285. I. Ninomiya, Heterocycles, 1974, 2, 105.

286. M.S. Premila and B.R. Pai, Ind. J. Chem., 1976, 14B, 134.

287. M.S. Premila, B.R. Pai and P.C. Parthasarathy, Ind. J. Chem., 1975, 13, 13; 945.

288. C. Casagrande and L. Canonica, J. Chem. Soc. Perkin I, 1975, 1647.

289. L. Cleaver, S. Nimgirawath, E. Ritchie and W.C. Taylor, Austr. J. Chem., 1976, 29, 2003.

290. Z. Horii, S. Uchida, Y. Nakashita, E. Tsuchida and C. Iwata, Chem. Pharm. Bull., 1974, 22, 583.

291. M. Shamma and D.-Y. Hwang, Tetrahedron, 1974, 30, 2279.

292. M. Hara, O. Hoshino and B. Umezawa, Chem. Pharm. Bull., 1976, 24, 262.

293. B. Umezawa and O. Hoshino, Heterocycles, 1975, 3, 1005.

294. S.M. Kupchan, O.P. Dhingra and C.-K. Kim, J. Org. Chem., 1976, 41, 4049.

295. S.M. Kupchan, A.J. Liepa, V. Kameswaran and R.F. Bryan, J. Amer. Chem. Soc., 1973, 95, 6861.

296. E.C. Taylor, J. G. Andrade and A. McKillop, Chem. Comm., 1977, 538.

297. T.R. Govindachari, B.R. Pai and G. Shanmugasundaram, Tetrahedron, 1964, 20, 2895.

298. K.L. Stuart, Heterocycles, 1976, 5, 701.

299. W.V. Curran, J. Heterocyclic Chem., 1973, 10, 307.

300. T. Kametani, T. Uryu and K. Fukumoto, J. Chem. Soc. Perkin I, 1977, 383.

301. S.M. Kupchan and C.-K. Kim, J. Amer. Chem. Soc., 1975, 97, 5623.

302. R. Gottlieb and J.L. Neumeyer, J. Amer. Chem. Soc., 1976, 98, 7108.

303. N.M. Mollov and H.B. Dutschewska, Tetrahedron Lett., 1966, 853.

304. S.M. Kupchan, T.-H. Yang, M.L. King and R.T. Borchardt, J. Org. Chem., 1968, 33, 1052.

305. H.H.A. Linde and M.S. Ragab, Helv. Chim. Acta, 1968, 51, 683.

306. M.P. Cava, A. Venkateswarlu, M. Srinivasan and D.L. Edie, Tetrahedron, 1972, 28, 4299.

307. M.P. Cava, D.L. Edie and J.M. Saá, J. Org. Chem., 1975, 40, 3601.

308. L. Castedo, R. Suau and A. Mourino, Heterocycles, 1975, 3, 449.

309. A.S. Orahovats, H.B. Dutschewska and N.M. Mollov, Dolk. Bolg. Akad. Nauk., 1973, 26, 491.

310. J.C. Sheehan and D.-D.H. Yang, J. Amer. Chem. Soc., 1958, 80, 1154.

311. T. Kametani, Y. Satoh and K. Fukumoto, Tetrahedron, 1973, 29, 2027.

312. J.M. Gulland and R.D. Haworth, J. Chem. Soc., 1928, 581.

313. T. Kametani, R. Nitadori, H. Terasawa, K. Takahashi and M. Ihara, Heterocycles, 1975, 3, 821.

314. M. Ohashi, J.M. Wilson, H. Budzikiewicz, M. Shamma, W.A. Slusarchyk and C. Djerassi, J. Amer. Chem. Soc., 1963, 85, 2807.

315. T. Kametani and K. Fukumoto, J. Chem. Soc., 1963, 4289.

316. T. Kametani, S. Shibuya, S. Seino and K. Fukumoto, Tetrahedron Lett., 1964, 25.

317. T. Kametani, H. Iida and C. Kibayashi, J. Heterocyclic Chem., 1970, 7, 339.

318. I. Noguchi and D.B. Maclean, Can. J. Chem., 1975, 53, 125.

319. H. Iida, T. Kikuchi, K. Sakurai and T. Watanabe, J. Pharm. Soc. Japan, 1969, 89, 645.

320. S. Ishiwata, T. Fujii, N. Miyaji, Y. Satoh and K. Itakura, Chem. Pharm. Bull., 1970, 18, 1850.

321. H. Iida, T. Kikuchi, S. Tanaka and M. Shinbo, J. Pharm. Soc. Japan, 1969, 89, 1169.

322. H. Iida, H.-C. Hsu, and T. Kikuchi, Chem. Pharm. Bull., 1973, 21, 1001.

323. H. Iida, H.-C. Hsu, T. Kikuchi and K. Kawano, Yakugaku Zasshi, 1972, 92, 1242.

324. H.-C. Hsu, T. Kikuchi, S. Aoyagi and H. Iida, Yakugaku Zasshi, 1972, 92, 1030.

325. T. Kametani, K. Fukumoto, and M. Fujihara, Chem. Pharm. Bull., 1972, 20, 1800.

326. T. Kametani and K. Fukumoto, Synthesis, 1972, 657.

327. A.H. Jackson and G.W. Stewart, Chem. Comm., 1971, 149.

328. A.H. Jackson, G.W. Stewart, G.A. Charnock and J.A. Martin, J. Chem. Soc. Perkin I, 1974, 1911.

329. T. Kametani, H. Iida, T. Kikuchi, M. Mizushima and K. Fukumoto, Chem. Pharm. Bull., 1969, 17, 709.

330. A.H. Jackson and G.W. Stewart, Tetrahedron Lett., 1971, 4941.

331. A.J. Birch, A.H. Jackson, P.V.R. Shannon and G.W. Stewart, J. Chem. Soc. Perkin I, 1975, 2492.

332. J. Niimi, Yakugaku Zasshi, 1960, 80, 451.

333. T.-L. Ho, Syn. Comm., 1974, 4, 351.

334. M.P. Cava and A. Afzali, J. Org. Chem., 1975, 40, 1553.

335. G. Hahn and K. Stiehl, Chem. Ber., 1938, 71, 2154.

336. O.N. Tolkachev, V.G. Voronin and N.A. Preobrazhenskii, Zhur. Obschei. Khim., 1958, 28, 3320.

337. A.R. Battersby, T.A. Dobson, D.M. Foulkes and R.B. Herbert, J. Chem. Soc. Perkin I, 1972, 1730.

338. W.W. Kaeding, J. Org. Chem., 1963, 28, 1063.

339. G. Hallmann and K. Hagele, Annalen, 1963, 662, 147.

340. W.G. Kofron and L.M. Baclawski, J. Org. Chem., 1976, 41, 1879.

341. A.G. Anderson, Jnr., and H.F. Greef, J. Amer. Chem. Soc., 1952, 74, 2923.

342. G.B. Bachman and R.W. Finholt, J. Amer. Chem. Soc., 1948, 70, 622.

343. I.M. Downie, J.B. Holmes and J.B. Lee, Chem. and Ind., 1966, 900.

344. E.J. Corey, C.U. Kim and M. Takeda, Tetrahedron Lett., 1972, 4339.

345. F.M. Hershenson, J. Org. Chem., 1975, 40, 740.

346. W. Steglich, V. Austel and H. Tanner, Chem. Ber., 1968, 101, 916.

347. J.V. Greenhill, Chem. Soc. Rev., 1977, 6, 277.

348. D. Smith and P.J. Taylor, Spectrochimica Acta, 1976, 32A, 1477.

349. D. Smith and P.J. Taylor, Spectrochimica Acta, 1976, 32A, 1503.

350. R.H.F. Manske, Can. J. Res., 1937, 15, 159.

351. G.F. Kirkbright, J.T. Stock, R.D. Pugliese and J.M.Bobbitt, J. Electrochem. Soc., 1969, 116, 219.

352. J.M. Bobbitt, K.H. Weisgraber, A.S. Steinfeld and S.G. Weiss, J. Org. Chem., 1970, 35, 2884.

353. J.M. Bobbitt, H. Yagi, S. Shibuya and J.T. Stock, J. Org. Chem., 1971, 36, 3006.

354. M. Tomita, K. Fujitani, Y. Masaki and K.-H. Lee, Chem. Pharm. Bull., 1968, 16, 1968.

355. B. Umezawa, O. Hoshino, H. Hara and J. Sakakibara, Chem. Pharm. Bull., 1968, 16, 381.

356. J.M. Bobbitt, J.T. Stock, A. Marchand and K.H. Weisgraber, Chem. and Ind., 1966, 2127.

357. A. Lovecy, R. Robinson and S. Sugasawa, J. Chem. Soc., 1930, 817.

358. A.R. Battersby, D.J. Lecount, S. Garratt and R.I. Thrift, Tetrahedron, 1961, 14, 46.

359. R. Robinson and S. Sugasawa, J. Chem. Soc., 1931, 3163.

360. E. Spath, A. Orechoff and F. Kuffner, Chem. Ber., 1934, 67B, 1214.

361. A. Brossi, A.I. Rachlin and S. Teitel, J. Heterocyclic Chem., 1967, 4, 417.

362. E. Spath and N. Polgar, Monatsh, 1929, 51, 190.

363. A. Kauffman and R. Radosević, Chem. Ber., 1916, 49, 675.

364. R.A. Robinson, J. Org. Chem., 1951, 16, 1911.

365. R. Dickinson, I.M. Heilbron and F. Irving, J. Chem. Soc., 1927, 1888.

366. I. Baxter, L.T. Allan and G.A. Swan, J. Chem. Soc., 1965, 3645.

367. J.M. Bobbitt and T.-T. Chou, J. Org. Chem., 1959, 24, 1106.

368. M. Tomita and H. Watanabe, J. Pharm. Soc. Japan, 1938, 58, 783.

369. T. Strukov, Zhur. Obschchei Khim., 1959, 29, 3831.

370. T. Strukov, Zhur. Obschchei Khim., 1961, 31, 2709.

371. K. Kratzl and G. Billek, Monatsh, 1952, 83, 1409.

372. N.K. Ushenko and T.E. Gorizdra, Ukranian Chem. J., 1950, 16, 545.

373. B. Helferich and H.-O.M. von Blumencron, Chem. Ber., 1953, 86, 1058.

374. T. Kametani and I. Noguchi, J. Chem. Soc. (C), 1969, 502.

375. G.J. Kapadia, Y.N. Vaishnav and M.B.E. Fayez, J. Pharm. Sci., 1969, 58, 1157.

376. M.T. Bogert and R.M Isham, J. Amer. Chem. Soc., 1914, 36, 514.

APPENDIX 1

Calculated Velocities of the Enzyme Induced Decarboxylations of Isoquinoline-1-Carboxylic Acids

acid	substrate conc. $[10^{-5}M]$	LACCASE initial velocities $[10^{-9}mol.min^{-1}]$ Run 1	LACCASE initial velocities Run 2	PEROXIDASE initial velocities $[10^{-9}mol.min^{-1}]$ Run 1	PEROXIDASE initial velocities Run 2
163	0.8	–	3.3	–	5.1
	1.7	5.1	5.7	6.9	6.9
	3.3	6.9	6.9	14.0	11.0
	6.7	9.3	10.0	23.0	14.0
	10.0	–	–	28.0	–
	13.0	14.0	14.0	33.0	21.0
	17.0	15.0	15.0	39.0	26.0
164	0.8	–	3.6	7.5	7.5
	1.6	5.7	6.3	15.0	15.0
	3.2	12.0	11.0	30.0	26.0
	6.3	18.0	16.0	57.0	45.0
	9.7	–	–	69.0	–
	13.0	25.0	27.0	87.0	72.0
	16.0	29.0	33.0	100.0	84.0
165* (Peroxidase runs carried out using $\frac{1}{2}$ volume of enzyme)	0.7	5.4	–	9.0	11.0
	1.3	8.1	7.5	23.0	22.0
	2.7	19.0	15.0	45.0	45.0
	5.3	39.0	30.0	96.0	93.0
	8.0	–	–	160.0	–
	11.0	69.0	51.0	200.0	180.0
	13.0	–	72.0	420.0	–
165	0.7			17.0	19.0
	1.3			48.0	39.0
	2.7			100.0	84.0
	5.3				170.0
166	0.5	0.9	–	4.2	4.2
	1.0	1.7	2.1	10.0	9.3
	2.0	4.2	3.9	22.0	19.0
	4.0	8.7	7.2	48.0	45.0
	6.0	–	–	72.0	–
	8.0	16.0	16.0	96.0	87.0
	10.0	20.0	21.0	120.0	110.0
194	1.3	0.6		0.6	
	2.7	1.1		0.8	
	5.3	2.2		1.3	
	11.0	3.9		3.0	
	13.0	4.5		3.6	