TRENT POLYTECHNIC

A STUDY OF THE OXIDATION OF SOME PHENOLIC AMINES

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DOCTOR of PHILOSOPHY

by

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PREFACE

The work described in this thesis was carried out by the author in the Department of Physical Sciences, Trent Polytechnic, Nottingham, between October 1972 and October 1976. A few compounds were obtained from other workers and acknowledgement is made in the text.

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

The author is grateful to Trent Polytechnic for the award of a Research Assistant Demonstratorship.

The work described in Section 2.3 has been published:

I.G.C. Coutts and M. Hamblin, *J.C.S. Perkin I*, 1975, 2445.

and also part of the work described in Section 3.2,

I.G.C. Coutts and M. Hamblin, *J.C.S. Chem. Commun.*, 1976, 58.

Michael R. Hamblin,
Trent Polytechnic,
October, 1976.

Hamblin

SUMMARY

The synthesis of nitrogen- and oxygen- containing spirodienones by the oxidation of amino- or hydroxy- substituted diphenylamines has been investigated. Preliminary attempts to prepare 2,4'-dihydroxy-N-acyl- (or N-tosyl)- diphenylamines by established methods gave poor results, but 2,4'-dialkoxy-N-tosyldiphenylamines were readily obtained from the reaction of aryl bromides with secondary sulphonamides under Ullmann conditions. The scope and limitation of this novel synthetic approach to a wide range of symmetrically and unsymmetrically substituted N-tosyl diphenylamines were explored, and among the tertiary sulphonamides thus accessible were N-2-aminophenyl-N-4-hydroxyphenyltoluene-4-sulphonamide, and a range of derivatives containing substituents on the amino group.

Oxidation of diphenolic and aminophenolic diphenylamine derivatives was investigated, and three spiroheterocycles containing novel ring systems were obtained; in the systems studied, the secondary toluene-4-sulphonamido group was found to undergo oxidative cyclisation as readily as a phenolic hydroxyl. The reaction of two of the dienones with methylmagnesium iodide gave anomalous products consistent with 1,3-addition of the Grignard reagent to the dienone carbonyl group.

Following publication of a report on anodic oxidations, a range of phenolic tetrahydroisoquinoline-1-carboxylic acids was prepared and found to undergo oxidative decarboxylation to dihydroisoquinolines when treated with enzymes known to oxidise phenols, namely fungal laccase and horseradish peroxidase. The

enzyme parameters (Km and V) for these reactions were determined. This reaction is additional evidence of the Hahn proposal for the biogenesis of isoquinoline alkaloids and also suggests that useful parallels may be drawn between enzymatic and electrochemical investigations.

7-O-Benzyl-2-formylcoclaurine was synthesised and subjected to controlled potential anodic oxidation. A product different from starting material was isolated but it could not be obtained completely pure.

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EXPERIMENTAL SECTION

ABBREVIATIONS

Bs	4-bromobenzenesulphonyl
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
DMF	N,N-dimethylformamide
DMSO	dimethylsulphoxide
EPR	electron paramagnetic resonance spectroscopy
ESR	electron spin resonance spectroscopy
Et	ethyl
HMPT	hexamethylphosphoric triamide
Me	methyl
NADPH	nicotinamide-adenine-dinucleotide phosphate (reduced form)
n.m.r.	^{1}H nuclear magnetic resonance spectroscopy
Ph	phenyl
TFA	trifluoroacetic acid
THF	tetrahydrofuran
THP	tetrahydropyranyl
t.l.c.	thin-layer chromatography
Ts	toluene-4-sulphonyl
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.

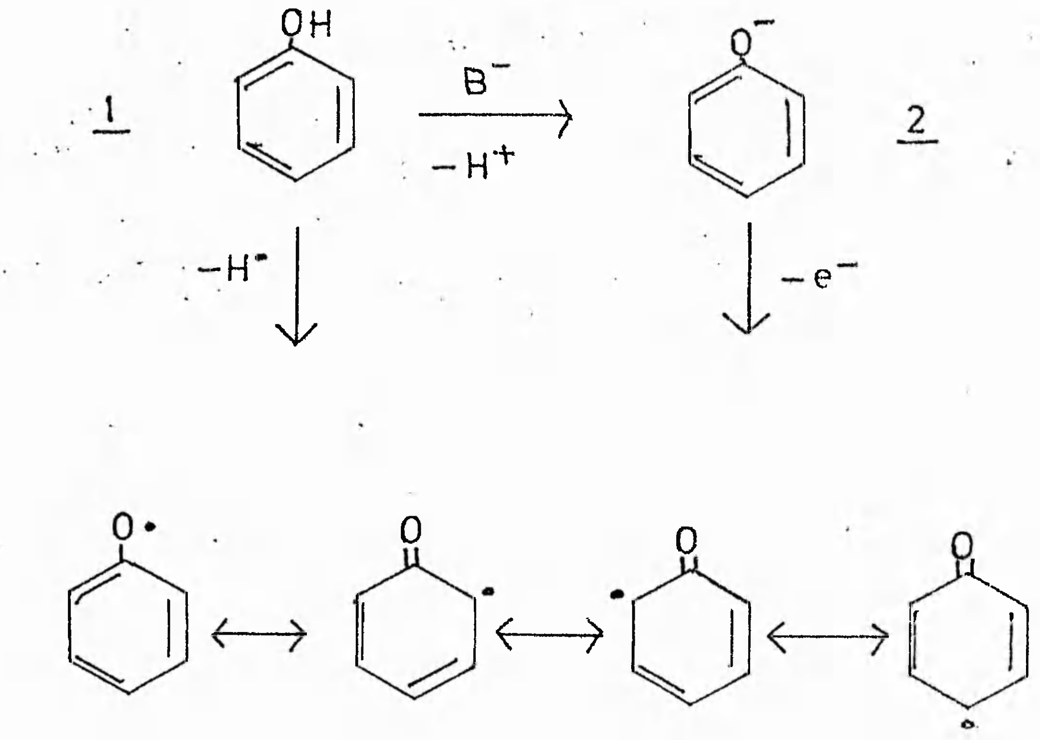
CHAPTER 1

INTRODUCTION

1.1 Phenol Oxidation

One of the characteristic chemical properties of phenols is their ready oxidation to compounds of different structural types by a large range of oxidants. The products are often complicated mixtures of compounds, dimeric, polymeric and quinonoid in nature. The wide occurrence of phenolic compounds in nature has led to many biosynthetic pathways incorporating oxidative coupling being proposed, and to attempts to duplicate the proposed coupling reactions _in vitro_. The field has been reviewed many times.[1-9]

Most work has been done with "one-electron oxidants", e.g. manganese dioxide, lead dioxide, silver oxide, ceric ions, ferric chloride and alkaline potassium ferricyanide, with the consequent proposal of a general mechanism involving generation of a phenoxyl radical by homolytic cleavage of the O-H bond in the phenol _1_ or by one electron oxidation of the phenoxide anion _2_. The phenoxyl radicals are resonance stabilised by delocalisation of the unpaired electron over the aromatic ring, _3a_-_d_. Although phenoxyl radicals with unsubstituted _ortho_ or _para_ positions usually undergo further reactions very rapidly,[10] due to the resonance stabilisation they have a longer lifetime than aryl or alkyl radicals and they do not attack the solvent or initiate polymerisation as readily.[4,7] Their transient existence has been confirmed by E.S.R. spectroscopy. Stable phenoxy radicals[12] are usually substituted in the 2, 4 and 6 positions by groups which



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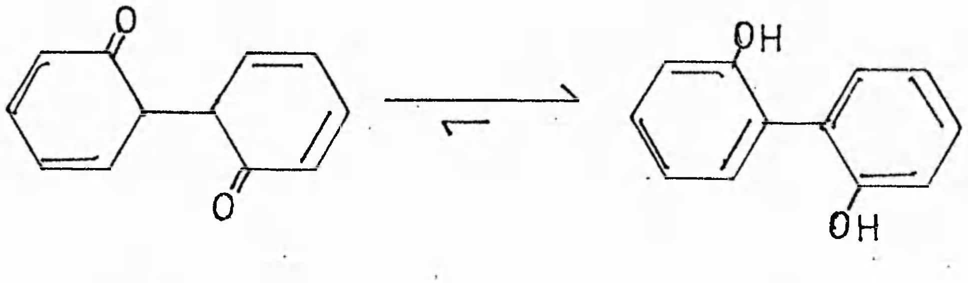
3b

3c

3d



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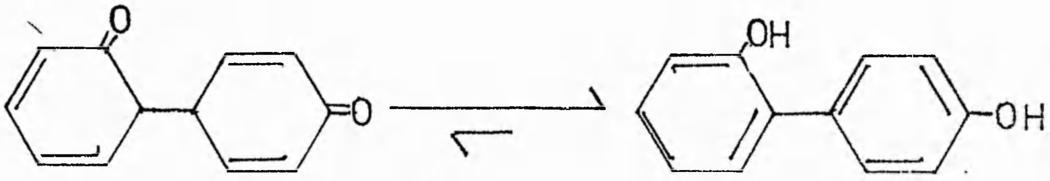

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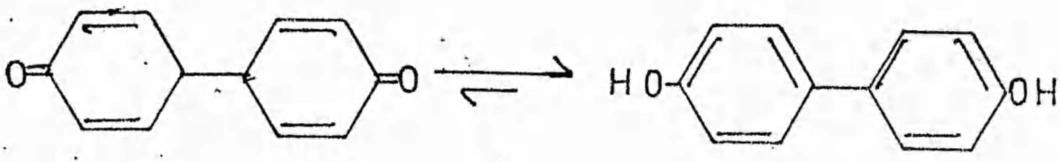
provide steric hindrance or increased delocalisation of the unpaired electron. Simple phenoxy radicals dimerise by either carbon-carbon or carbon-oxygen bond formation to give initial products 4-8 which aromatise in protic solvents to the more stable tautomers 9-13. In the case of the p-p coupled dimer 14, further oxidation often occurs[13] to give an extended quinone 15.

Recently, alternative mechanisms have been proposed; e.g. Barton[14] gives the following:-

1. $\mathrm{ArO\cdot + ArO\cdot \longrightarrow Dimer}$
2. $\mathrm{ArO\cdot + Ar\dot{O}H^{+} \longrightarrow Dimer + H^{+}}$
3. $\mathrm{Ar\dot{O}H^{+} + Ar\dot{O}H^{+} \longrightarrow Dimer + 2H^{+}}$
4. $\mathrm{ArOH + ArO^{+} \longrightarrow Dimer + H^{+}}$
5. $\mathrm{ArO^{-} + ArO^{+} \longrightarrow Dimer}$
6. $\mathrm{ArO\cdot + ArOH \longrightarrow Dimer + H\cdot}$
7. $\mathrm{ArO\cdot + ArO^{-} + H^{+} \longrightarrow Dimer + H\cdot}$
8. $\mathrm{ArO\cdot + ArO^{+} + e^{-} \longrightarrow Dimer}$

Reactions 1-3 correspond to simple coupling of radicals in different states of protonation. Reactions 6-8 are substitution reactions of phenoxyl radicals. Reaction 6 is thought improbable[15] because substitution of phenoxyl radicals into the anisole ring does not take place, and anisole and phenols should be equivalent with respect to radical substitution. Reaction 7 is not rejected outright as evidence has been brought for its occurrence.[16,17] Reaction 8 is dismissed[14] as involving the collision of "two improbable particles". Reactions 4 and 5 involve phenoxonium cations for which many workers have tried to find evidence.

A benzoyloxy cation has been reported[18] as an intermediate in the oxidative cyclisation of biphenyl-2-carboxylic acid with chromic acid and with hydrogen peroxide. Stable salts of



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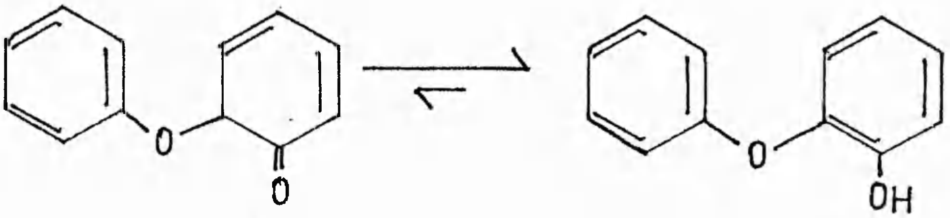

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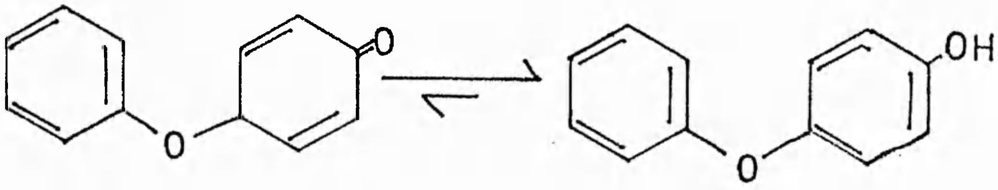

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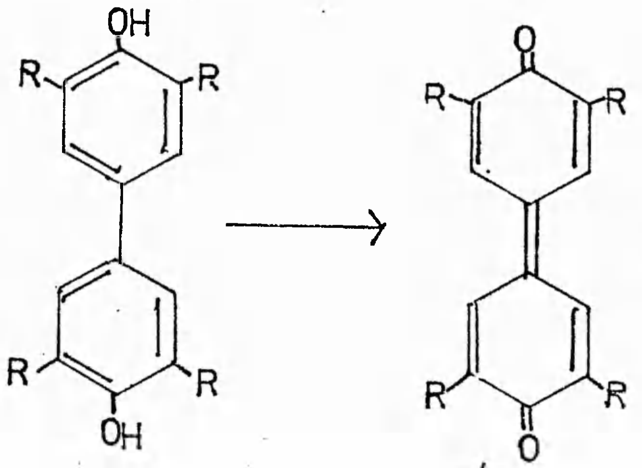

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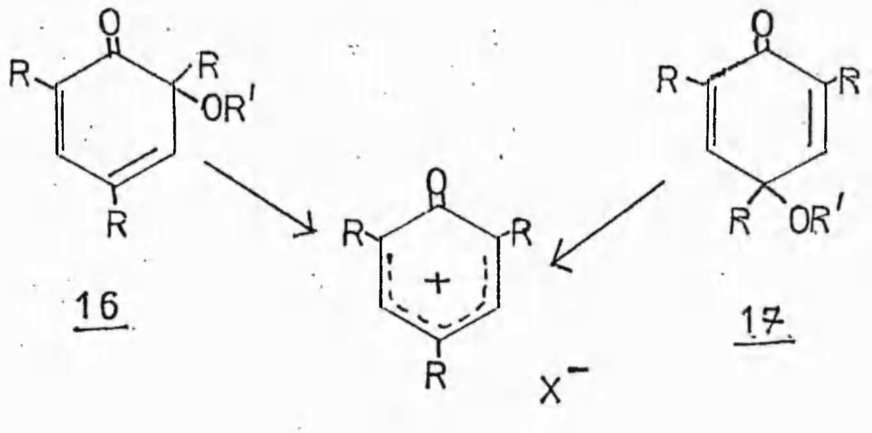
phenoxonium ions <u>18</u> have been prepared[19] by decomposing the quinol ethers <u>16,17</u> (R = C_6H_5 or 4-MeO-C_6H_4; R$'$ = Me, Et or Ac) with boron trifluoride or antimony pentachloride. The cyclisation of 2-hydroxy-3$'$-methoxybenzophenones to the corresponding xanthones by DDQ was thought[20] to proceed via phenoxonium ions. Waters[21] proposed that carbon-oxygen-carbon coupling was a radical process but that carbon-carbon coupling was likely to be ionic, because a phenoxyl radical has more of the unpaired electron density concentrated at the oxygen atom, while phenoxonium ions exist as mesomeric carbonium ions. Many reports state that phenoxonium-type intermediates occur when a metal oxidant forms an initial complex with a phenol and this complex undergoes heterolysis leaving a positive charge on the aromatic ring. Oxidants of this type include vanadium oxytrichloride[22], lead dioxide and lead tetra-acetate[23], and manganese tris(acetylacetonate).[24] Thallium trifluoroacetate has also been suggested[25] to be a reagent which produces incipient phenoxonium ions, but a recent paper[26] indicates that thallic salts react by ipso-thallation of the aromatic ring followed by nucleophilic displacement of the thallium.

Anodic oxidation has produced phenoxonium ions. Thus oxidation of 2,6 di-t-butyl-4-methylphenol gave products arising from quenching of the phenoxonium ion by nucleophiles,[27] while the oxidation of monophenolic diphenylpropanes was found[28] to proceed through electrophilic attack of phenoxonium ions on the unoxidised ring. The anodic oxidation of an α-tocopherol produced a phenoxonium ion <u>19</u> of remarkable stability.[29]



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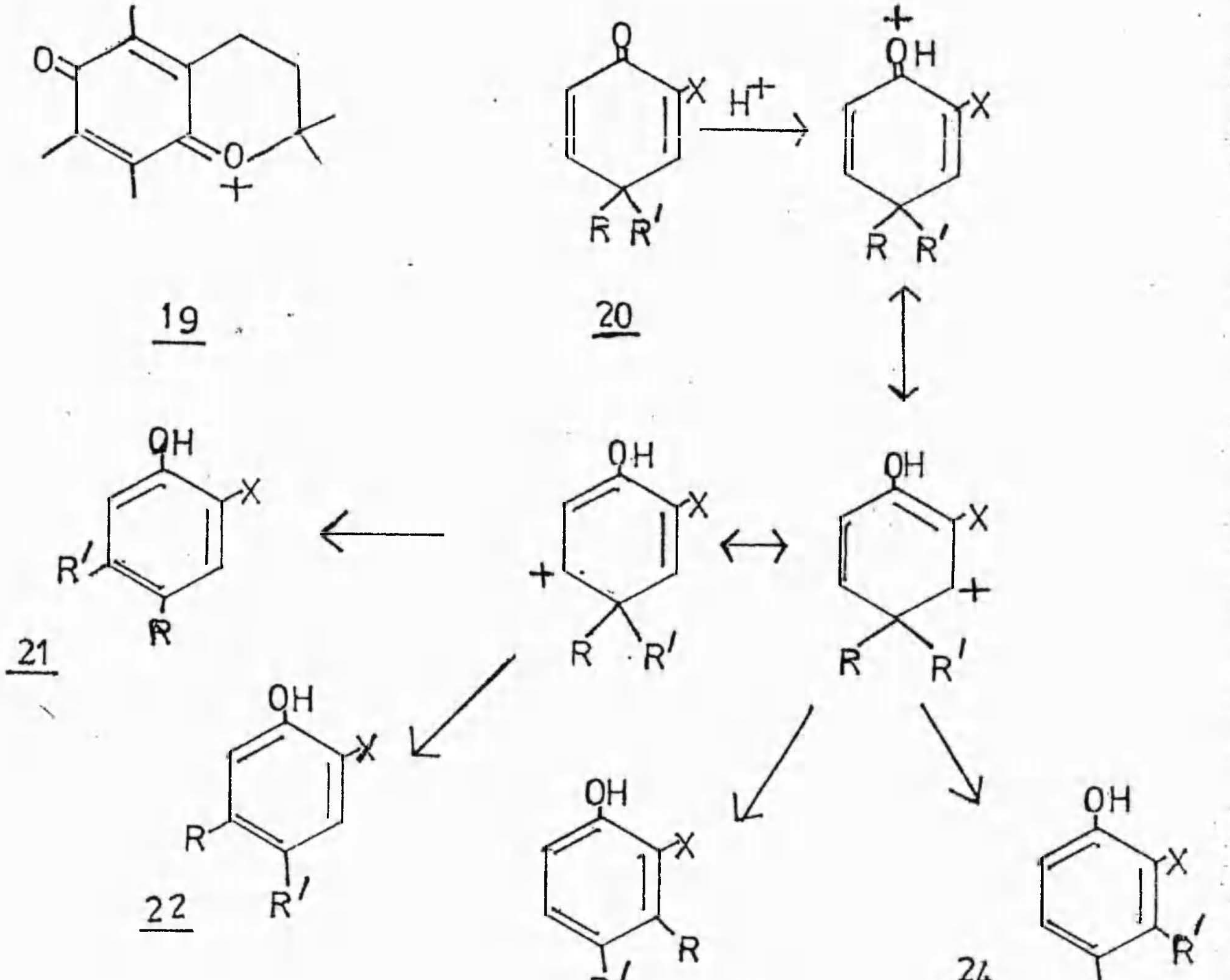

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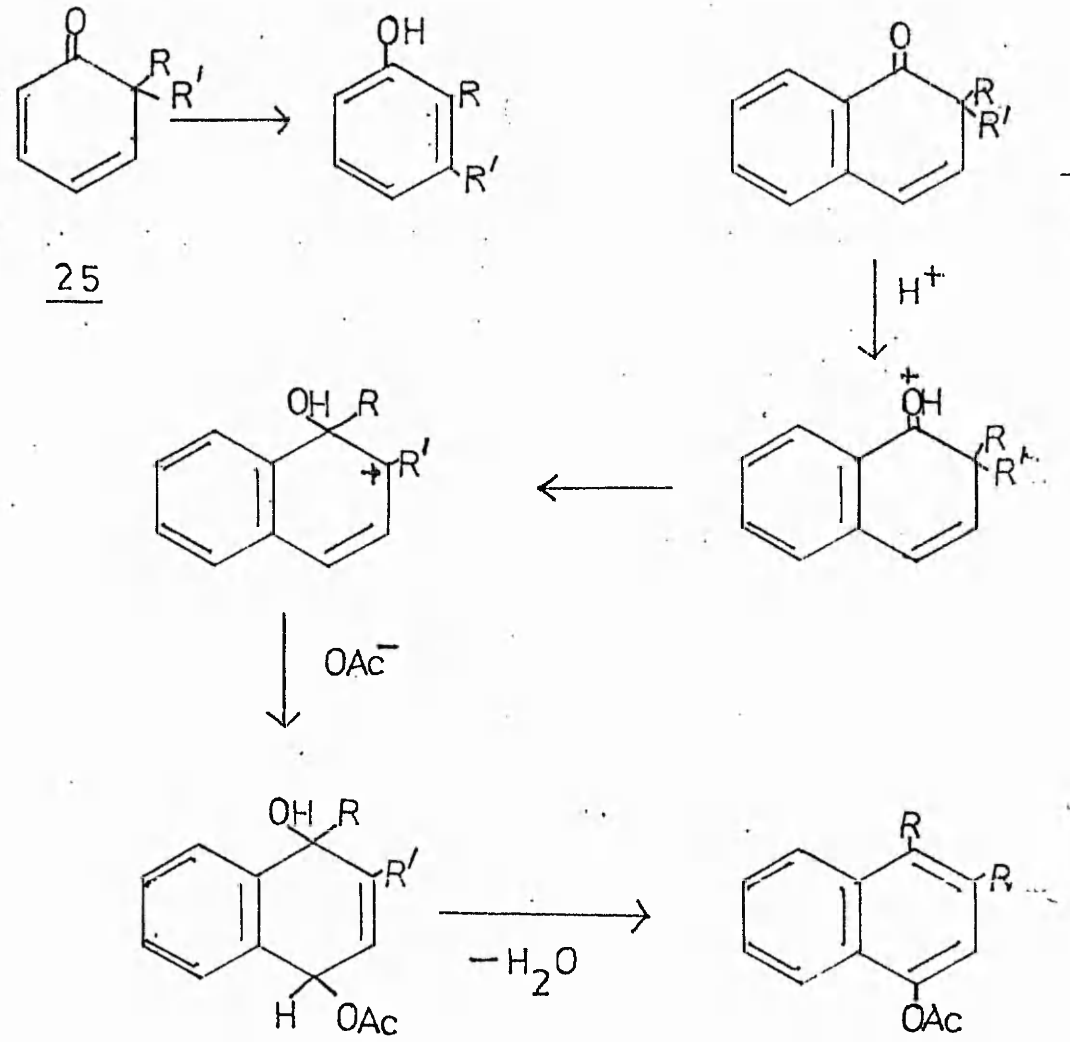
1.2 The Dienone-Phenol Rearrangement

This is an acid-catalysed carbonium ion rearrangement. In the case of the 2-substituted cyclohexa-2,5-dienone 20 there are four possible products 21-24. The precise product formed cannot be predicted with certainty. The effect of the 2-substituent can be steric, e.g. a tert-butyl group would tend to direct the rearrangement toward C_5 rather than C_3. An electronegative group such as bromine would destabilise an intermediate with the positive charge near to it and produce a similar effect.[30] Some qualitative effects of the nature of R^1 and R^2 have been determined. Normally a more substituted alkyl group migrates more readily than a less substituted one[31] and phenyl groups migrate in preference to alkyl groups.[32-35] A phenyl group substituted with a -M group migrates less readily than an unsubstituted or +M substituted one.[36] In all cases a carbon bond migrates more readily than a heteroatom bond.

Cyclohexa-2,4-dienones can react by two mechanistic paths. Monocyclic compounds 25 react mainly by normal 6-5 migration,[37] with the same general effects of the nature of R as for cyclohexa-2,5-dienones. In the case of bicyclic compounds, e.g. the naphthalene derivatives 26, the reaction proceeds as shown, with the attack of a nucleophile (usually acetate when the rearrangement occurs in acetic anhydride-sulphuric acid) at the 4-position.[38]

A closely related reaction is the dienol-benzene rearrangement which also proceeds through a carbonium ion 27 and like the dienone-phenol rearrangement is thought to be an important step in



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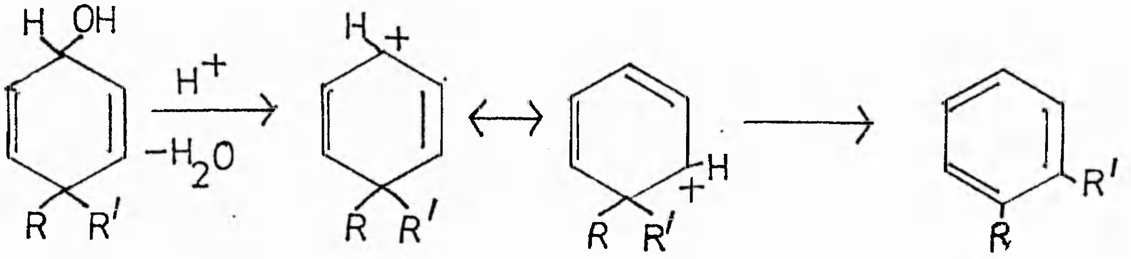

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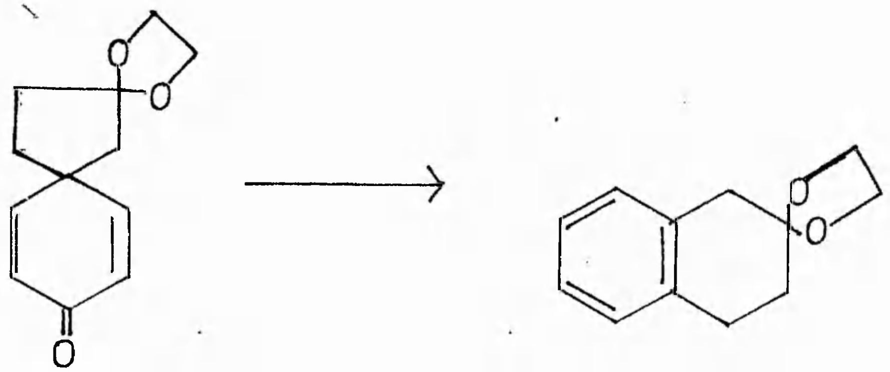

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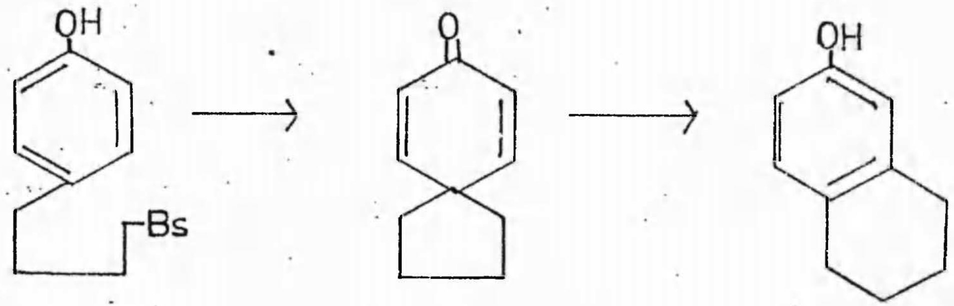
the biosynthesis of natural products. Cyclohexadienones can be reduced to dienols by sodium borohydride or the Meerwein-Verley-Ponndorf method. Thus the spirodienone 28 on treatment with aluminium iso-propoxide gave the tetrahydronaphthalene derivative 29, the intermediate dienol having undergone spontaneous rearrangement.[39]

1.3 A Review of the Synthesis and Rearrangement of Carbocyclic Spirocyclohexadienones

1.3.1 Aryl participation routes

Aryl participation reactions[40] are a general class described by the abbreviation Ar_n-X where n refers to the position on the aromatic ring and X the number of atoms in the side chain involved in the cyclisation. As yet they have only been used to produce spirocyclohexa-2,5-dienones. Baird and Winstein[41] described the first example, an Ar_1-5 reaction in which the brosylate 30 was treated with potassium t-butoxide to form the spirodienone 31 in 50% yield. The product underwent a quantitative dienone-phenol rearrangement to 32. In a later paper[42] they isolated the unstable spirodienone 34 in very small yield from an Ar_1-3 reaction induced by passing the bromide 33 down a column of basic alumina. Russian workers[43-46] have prepared di-t-butyl spirodienones 35 (R^1 and R^2 = alkyl or aryl) in 55-85% yields and 36 (R^1 and R^2 = alkyl or cyclohexyl) in 20-80% yields by treatment of the appropriate ethyl tosylates with potassium t-butoxide.

Ar_1-5 cyclisations have been reported by many workers. The spirodienone 38 was prepared[39] in 40% yield either by treatment



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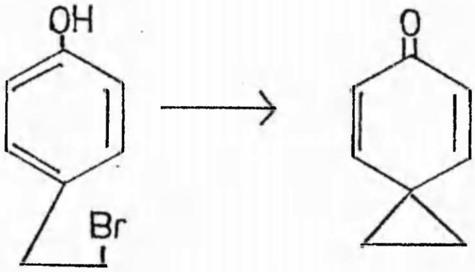

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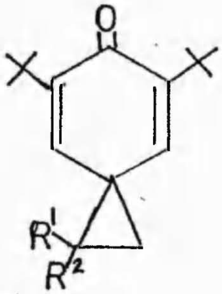

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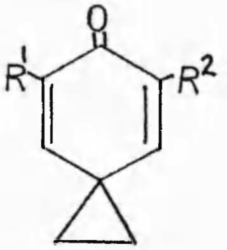

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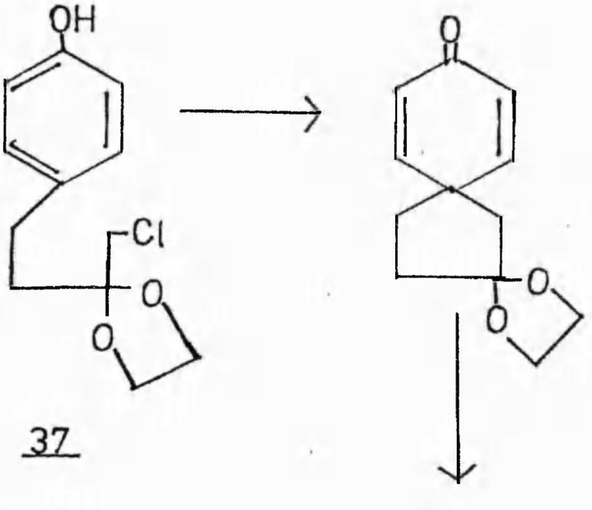

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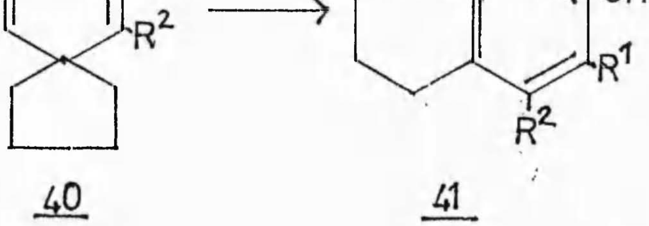

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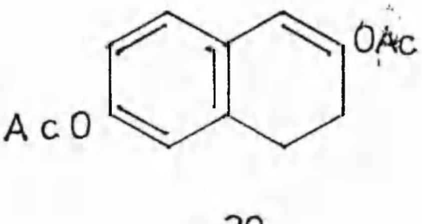

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of the chloride 37 with t-butoxide, or by pyrolysis of the sodium salt of 37. The dienone underwent rearrangement in acetic anhydride-sulphuric acid to 2,6-diacetoxy-3,4-dihydronaphthalene 39, while 70% acetic acid only hydrolysed the ketal function. The 2- and 3- substituted spirodienones 40 (R^1 and R^2 = H, Me and OMe) have been prepared[47,48] in 40-80% from the corresponding ethyl brosylates, and rearranged in methanol containing sulphuric acid to 41. When the substituent was in the 2-position migration occurred exclusively to the 5-position for steric reasons. The bicyclospirodienone 43 was prepared[49] in 8% yield from the tosylate 42. Other Ar_1-5 cyclisations have been reported.[50,51]

Ar_1-5 routes have been used in approaches to the synthesis of natural products. Masamune[52] prepared the dienone 45 from the bromide 44 as a key intermediate in total syntheses of the terpene kaurene and the terpene alkaloid atisine. Two groups of workers[53,54] simultaneously reported syntheses of the sesquiterpene cedrene using the spirodienone 46 (R = Me or Et) as an intermediate. The dienone 48, prepared from 47, has been used[55] in an approach to the synthesis of the terpenoid β-vetivone. On treatment with ethanolic hydrochloric acid the dienone underwent rearrangement to phenolic alcohol 49 or 50 but the exact structure of the product was not determined.

An Ar_1-6 cyclisation was reported[56] in 1957. The potassium salt of the phenol 51 was converted into the spirodienone 52 by heating in dilute solution. An extension of this method[57] gave

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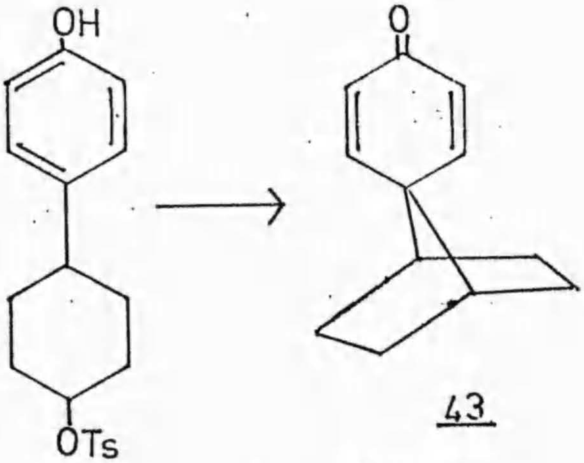

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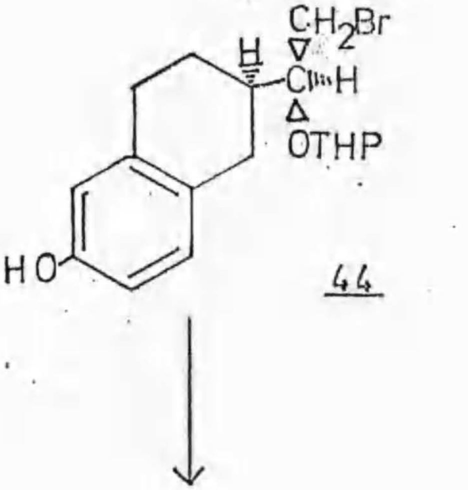

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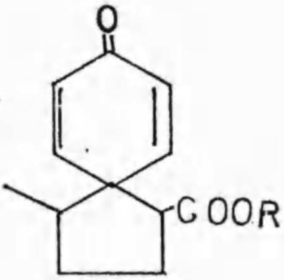

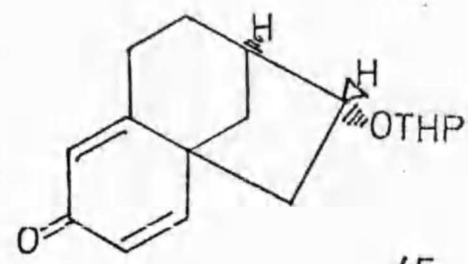

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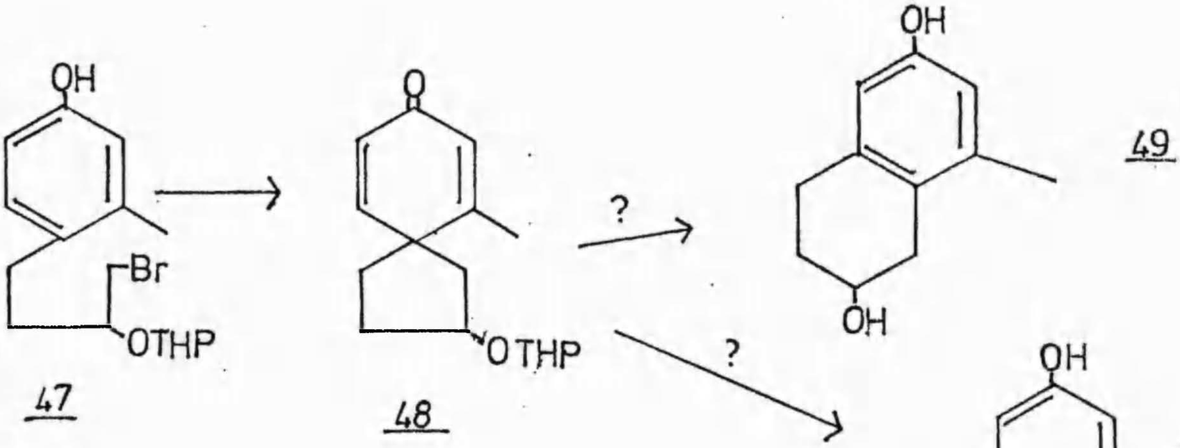

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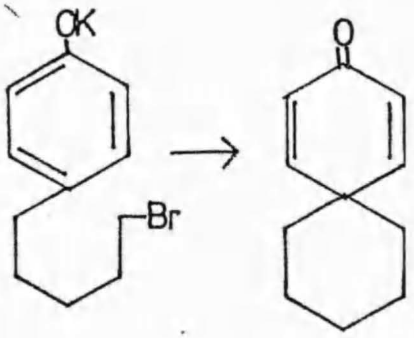

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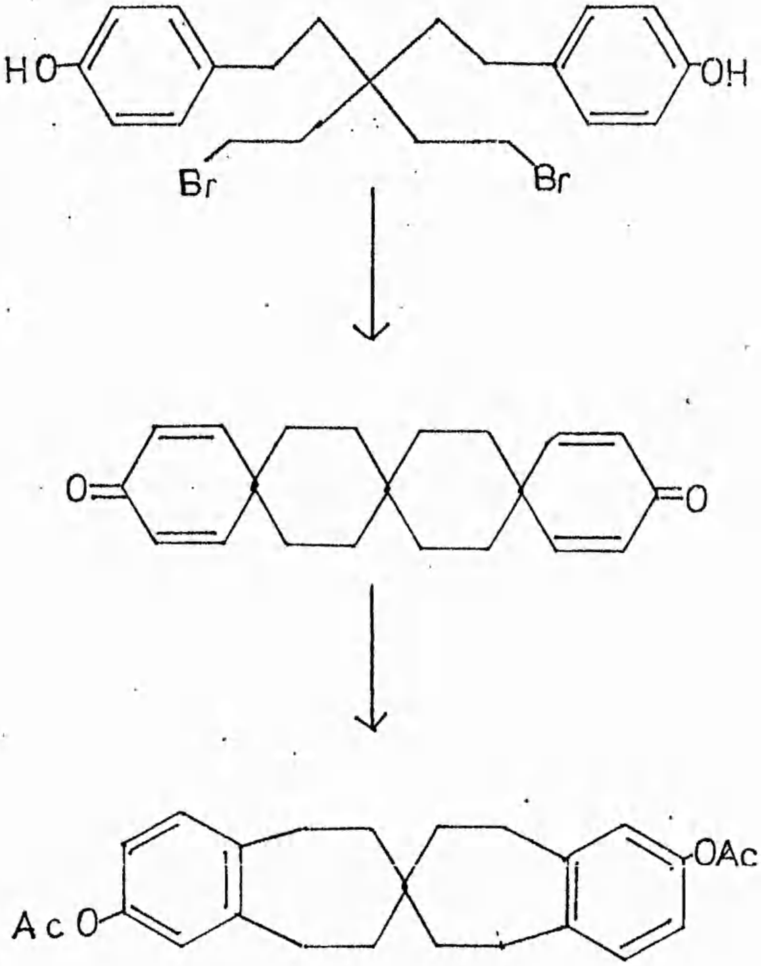
a 70% yield of 54 from 53. This bis-spirodienone on treatment with acetic anhydride containing a trace of sulphuric acid underwent a double dienone-phenol rearrangement to give the diacetoxybibenzosuberane 55. The quantitative conversion of the di-t-butyl tosylate 56 into the dienone 57 has been attributed[58] to steric hindrance around the phenolic oxygen cutting down intermolecular reactions. Beames and Mander[59] used an Ar_1-6 cyclisation in an approach to the synthesis of the diterpene alkaloid hetisine. They obtained 59 as a mixture of epimers in 50% yield by heating 58 with t-butoxide. The same authors[60] introduced a new method of carrying out Ar_1 cyclisations involving treatment of phenolic diazoketones with catalytic quantities of acids whose conjugate bases are poor nucleophiles (e.g. fluoroboric acid) in high dielectric constant solvents (e.g. nitromethane). They prepared 61 from 60 in 65% yield.

1.3.2 Phenol Oxidation

Three membered rings have been formed by oxidation of phenols. Treatment of the bisphenol 62 in benzene solution with alkaline ferricyanide[61,62] gave the bis-spirodienone 63. The analogous compound 65 was obtained[63] in 100% yield by shaking the bisphenol 64 in benzene with manganese dioxide for 20 minutes. It has been determined[64] by E.S.R. studies that the bis-spirodienone 67 (obtained from the phenol 66) is in equilibrium with the biradical 68. Larger rings have been obtained by the oxidation of



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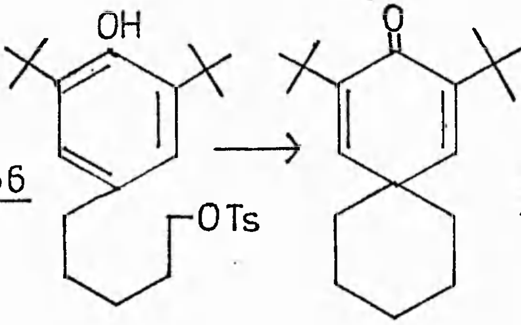

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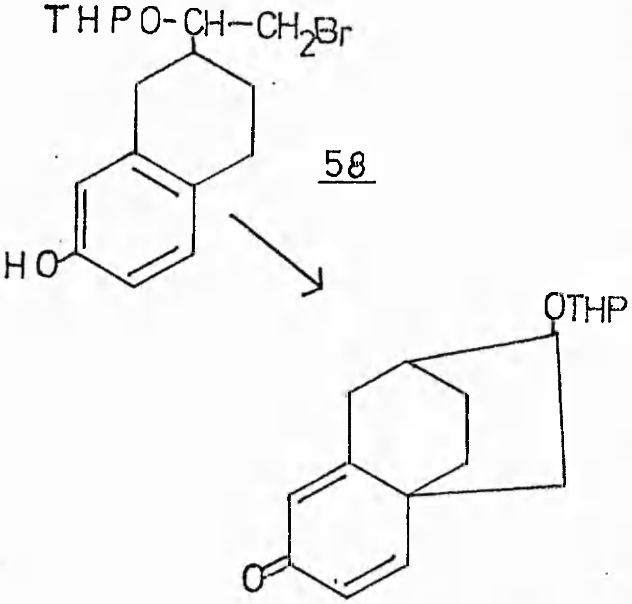

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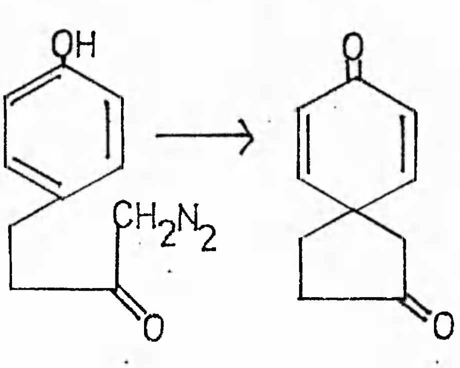

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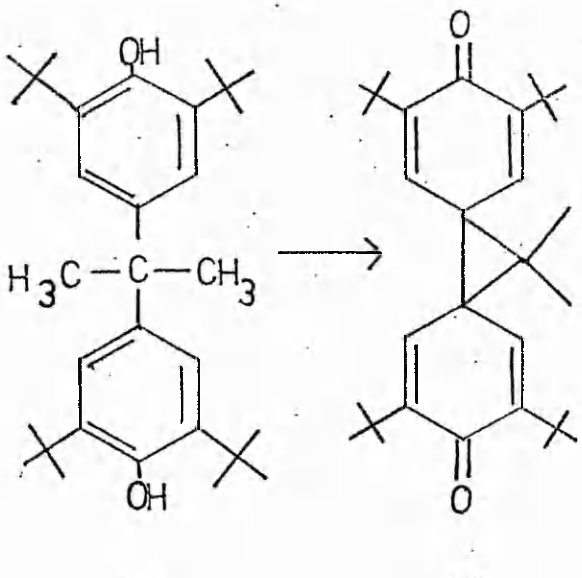

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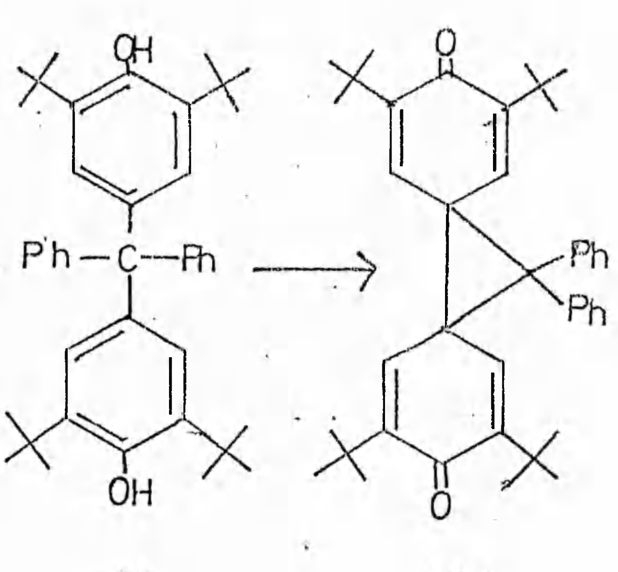

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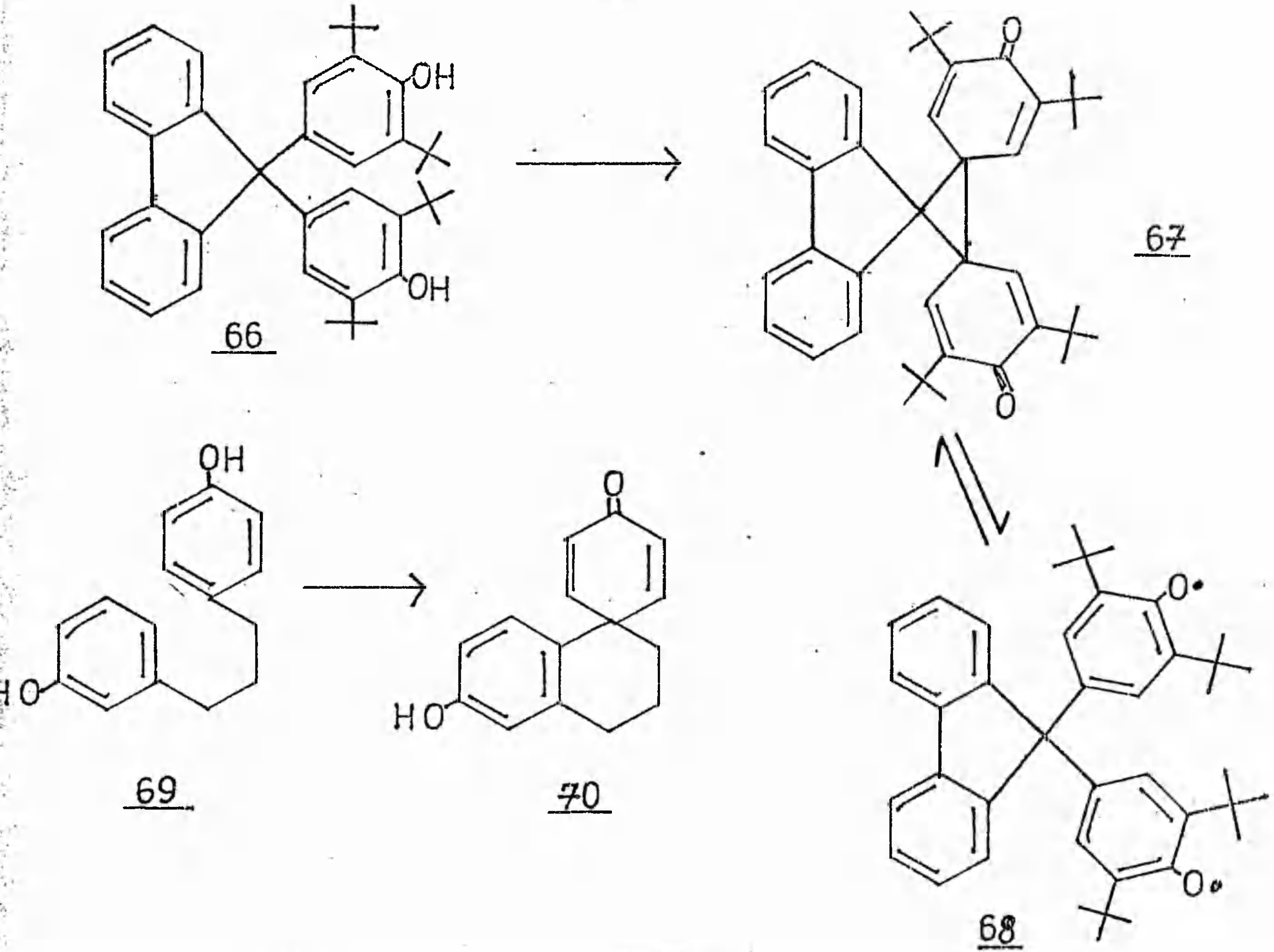


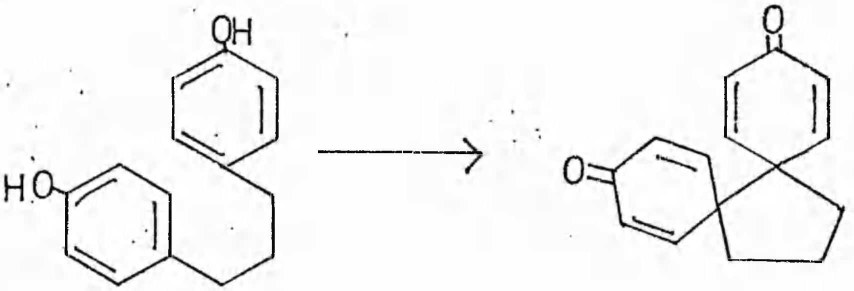
66 67 68 69 70

dihydroxydiphenylalkanes. Treatment of the dihydroxydiphenylpropane 69 with vanadium oxytrichloride gave a 76% yield of the spirodienone 70, in contrast to the low yields obtained with other oxidants;[65] this was attributed to the formation of a vanadium complex which aided intramolecular coupling. Similarly, two-phase ferricyanide oxidation,[66] which also favours intramolecular coupling, converted the bisphenols 71 and 73 to the bisdienones 72 and 74 in 20 and 15% yields respectively. Oxidation[67] of the diphenolic diphenylpropanes 75, 77 and 79 with a novel complex reagent obtained from ferric chloride and DMF gave the spirodienones 76, 78 and 80 in 39, 67 and 67% yields.

In 1973 the oxidation of the monophenol 81 to 82 by thallium trifluoroacetate was reported;[25] the anodic oxidation of this system was studied the following year.[28] The monophenolic diphenylpropanes 83, 85 and 87 were cyclised to the spirodienones 84, 86 and 88 in high yields. The authors[28] reported a dienone-phenol rearrangement to 89 with trifluoroacetic acid but give no structural proof of their product. It is perhaps odd that alkyl rather than aryl migration has occurred.[32]

1.3.3 Rearrangement Routes

Various rearrangements have resulted in the formation of carbocyclic spirodienones. Thus the acetal 90 gave the dienone 92 in 60% yield on treatment with sulphuric acid in acetic acid, a reaction which was assumed[68] to proceed by a pinacol rearrangement of the intermediate diol 91. The spirodienone 92 was also



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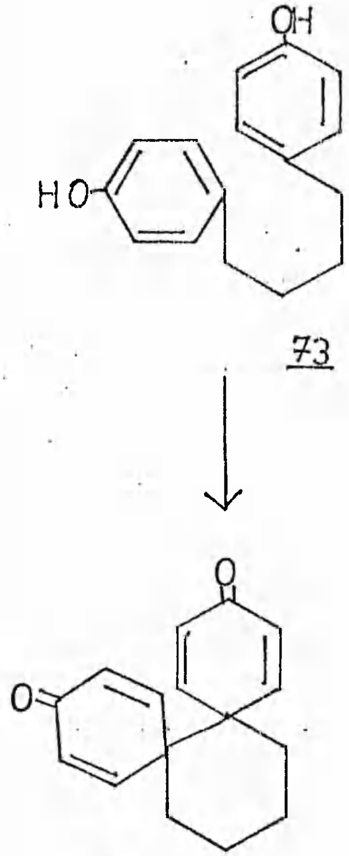

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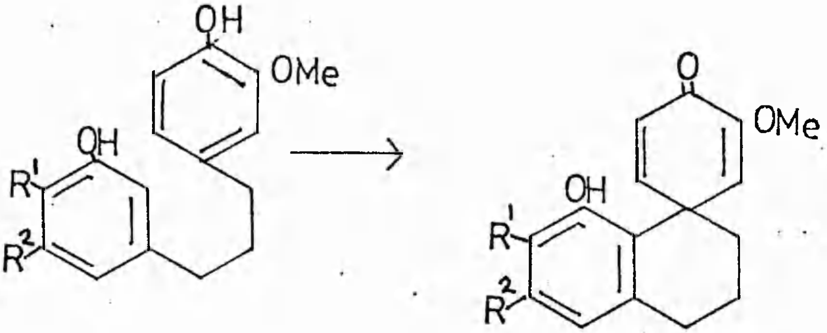
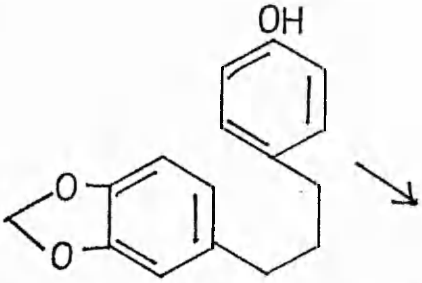


75 $R^1 = R^2 = H$ 76

77 $R^1 = OMe$, $R^2 = H$ 78

79 $R^1 = R^2 = OMe$ 80



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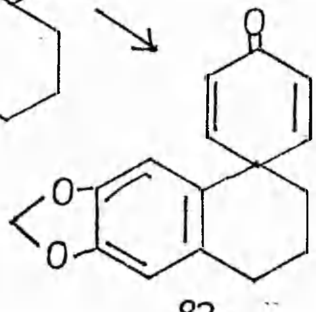

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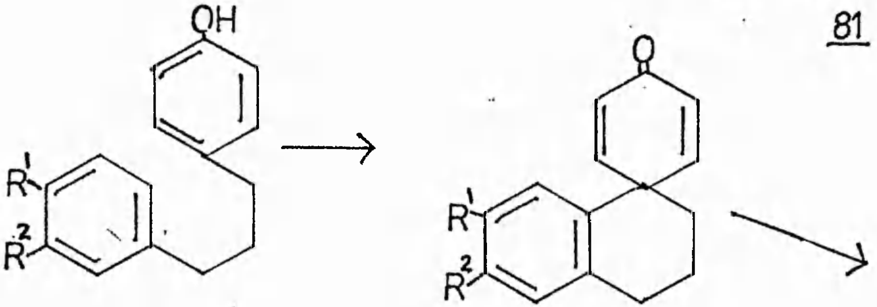
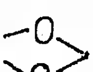
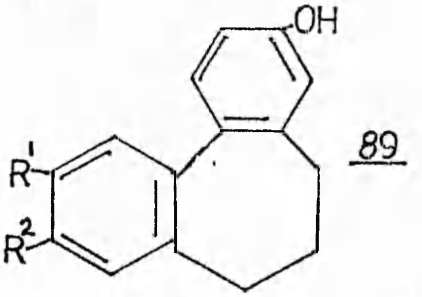


83 $R^1 = H$, $R^2 = OMe$ 84

85 $R^1 = R^2 = OMe$ 86

87 $R^1 R^2 =$  88



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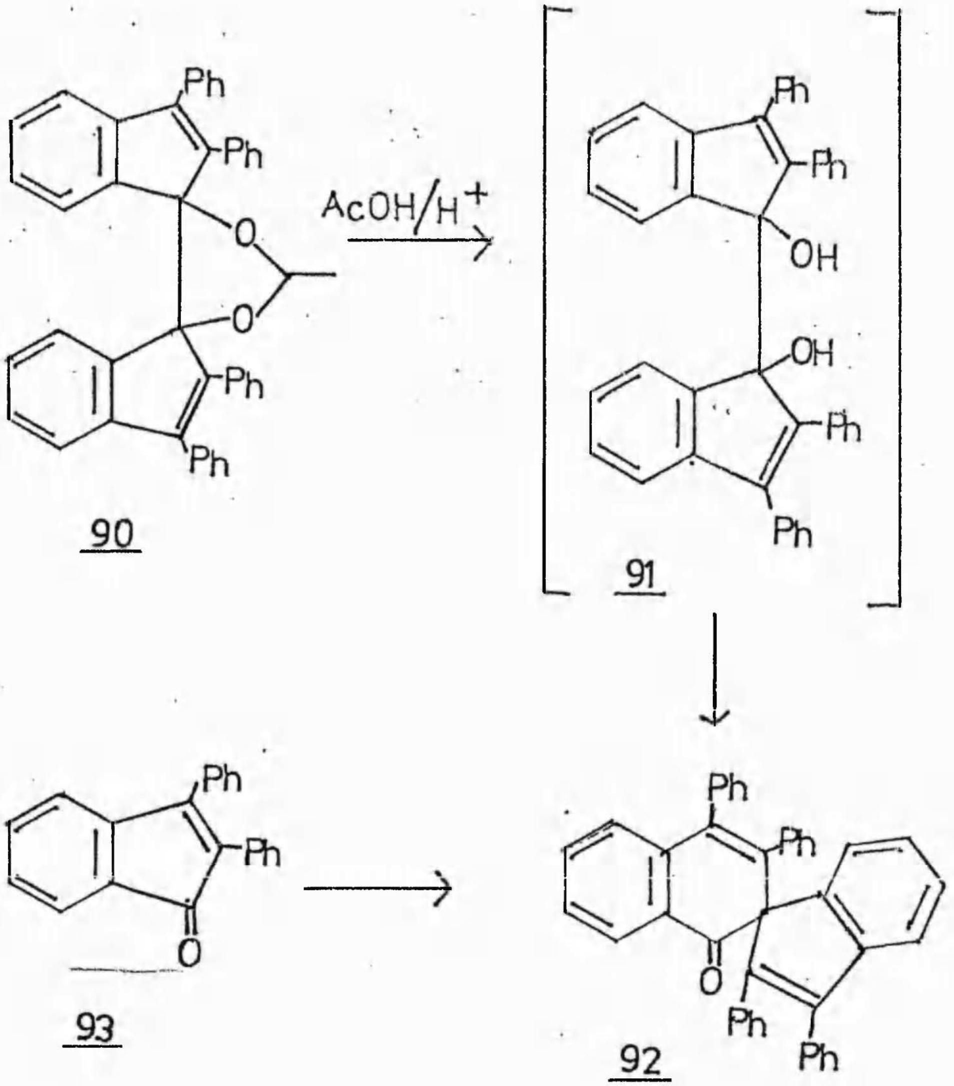
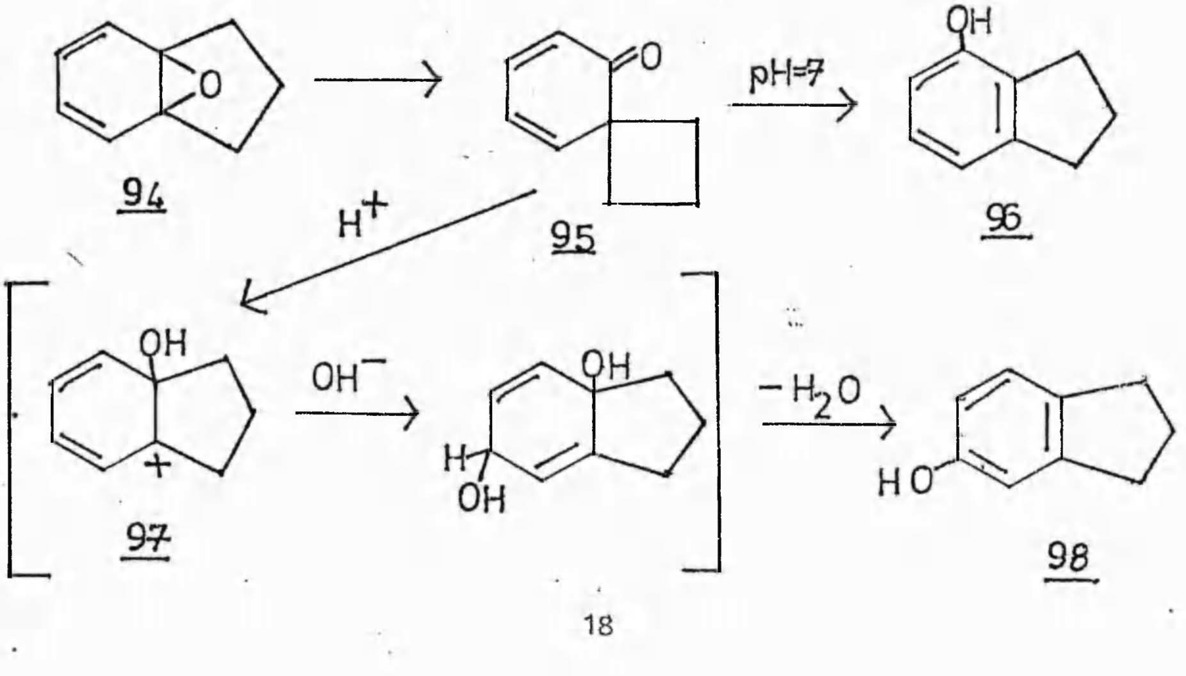

Ph
AcOH/H+
O
OH
90
91
93
92


O
pH=7
OH
H+
94
95
96
OH
OH−
H
−H2O
HO
97
98

formed in 38% yield by treatment of the indenone 93 with zinc dust and acetyl chloride. Acid catalysed rearrangement[69] of the oxirane 94 led to the spirodienone 95 which rearranged in neutral or basic conditions to 4-hydroxyindane 96, but in acid 32% of 5-hydroxyindane 98 was formed presumably *via* the carbonium ion 97. An analogous reaction is the production[70] of 100 by thermolysis of the oxirane dimer 99 at 420°. The product was reported to undergo a ready dimerisation.

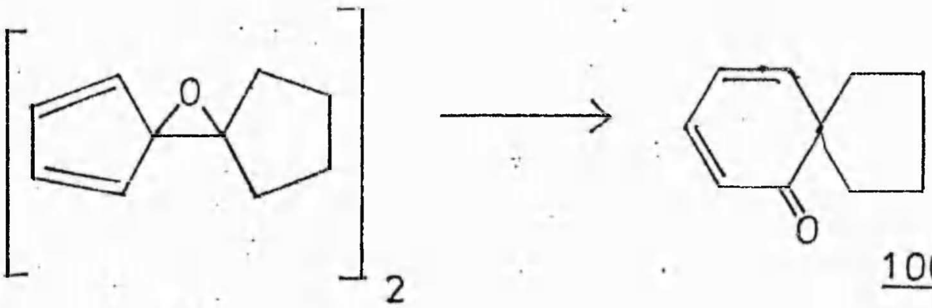
When the hydrocarbon 101 was oxidised[71] with trifluoroperacetic acid and boron trifluoride etherate at 0° the dienone 102 was obtained; if the reaction was carried out at -65° a mixture of 102 and 103 was obtained.

The photochemical rearrangement of O-acetyldehydrotestosterone 104 has been reported by several workers[72-74] to give spirodienones among other products. Dutler *et al*[74] isolated 105 in 18% and 106 in 1% yields from photolysis in dioxan. The products underwent dienone-phenol rearrangements in acetic anhydride-sulphuric acid, to give aromatic steroid derivatives; 105 gave 107 and 106 gave 108 in good yields. Both these rearrangements are examples of the more substituted alkyl group migrating preferentially.

Photochemical rearrangements of bicyclic enones have given spirodienones. Thus 110 was prepared from 109 in low yield by irradiation of an aqueous acetic acid solution,[75] while 111 was converted to 112 by a similar method.[76]

1.3.4 Miscellaneous Methods

Before the discovery of the Ar_1 participation route carbocyclic



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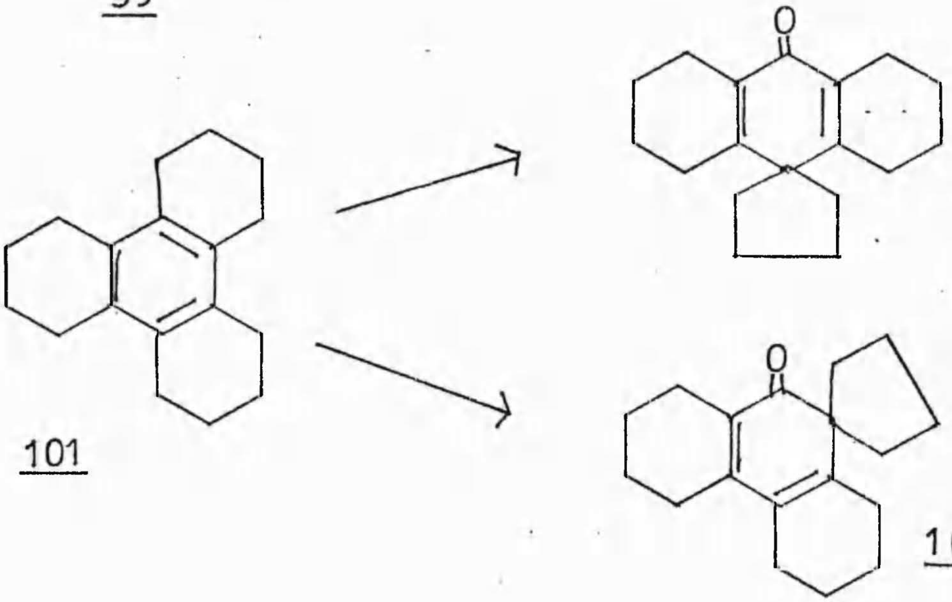

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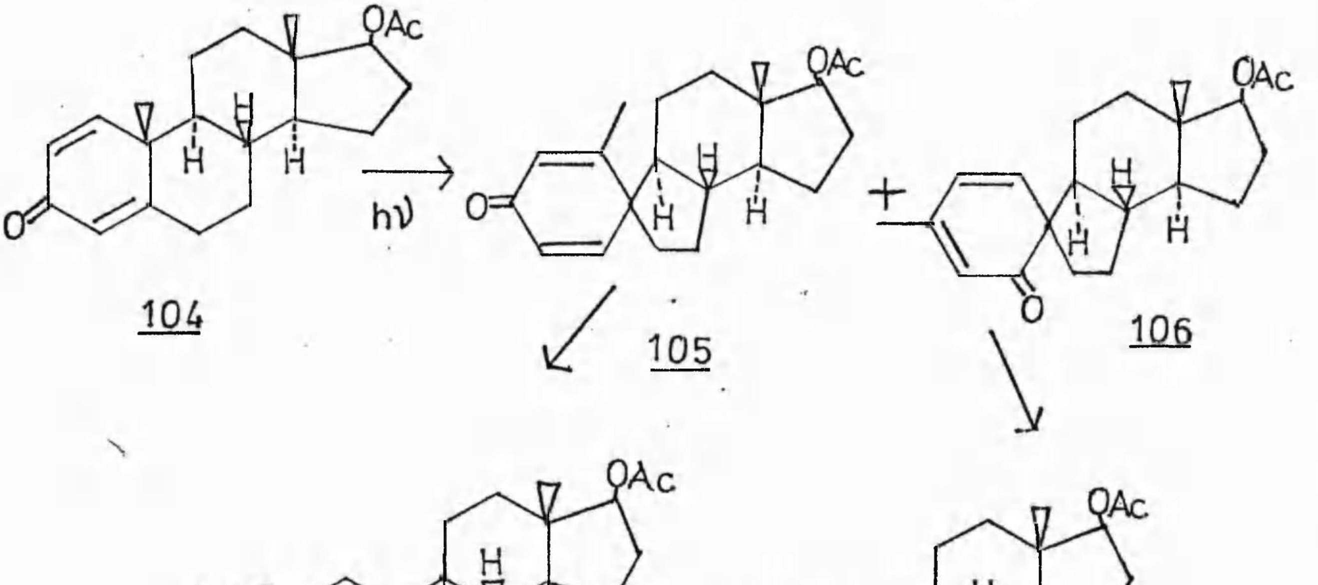
102

103



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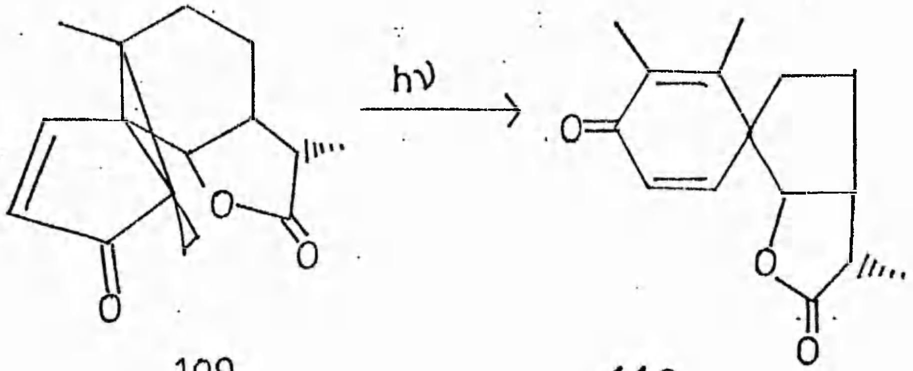

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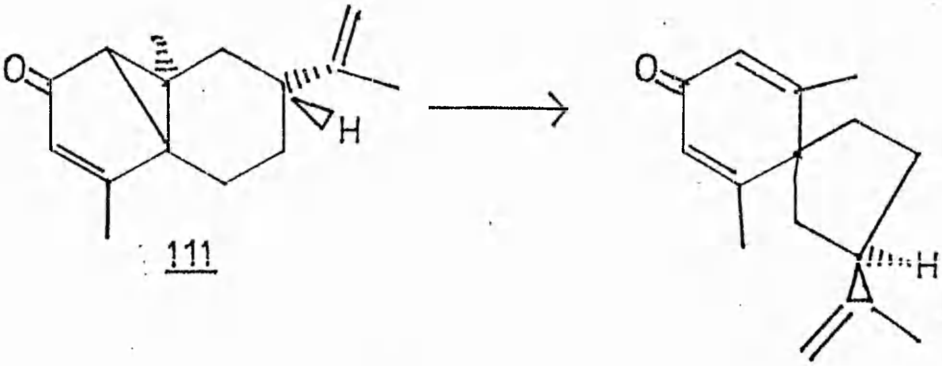
110



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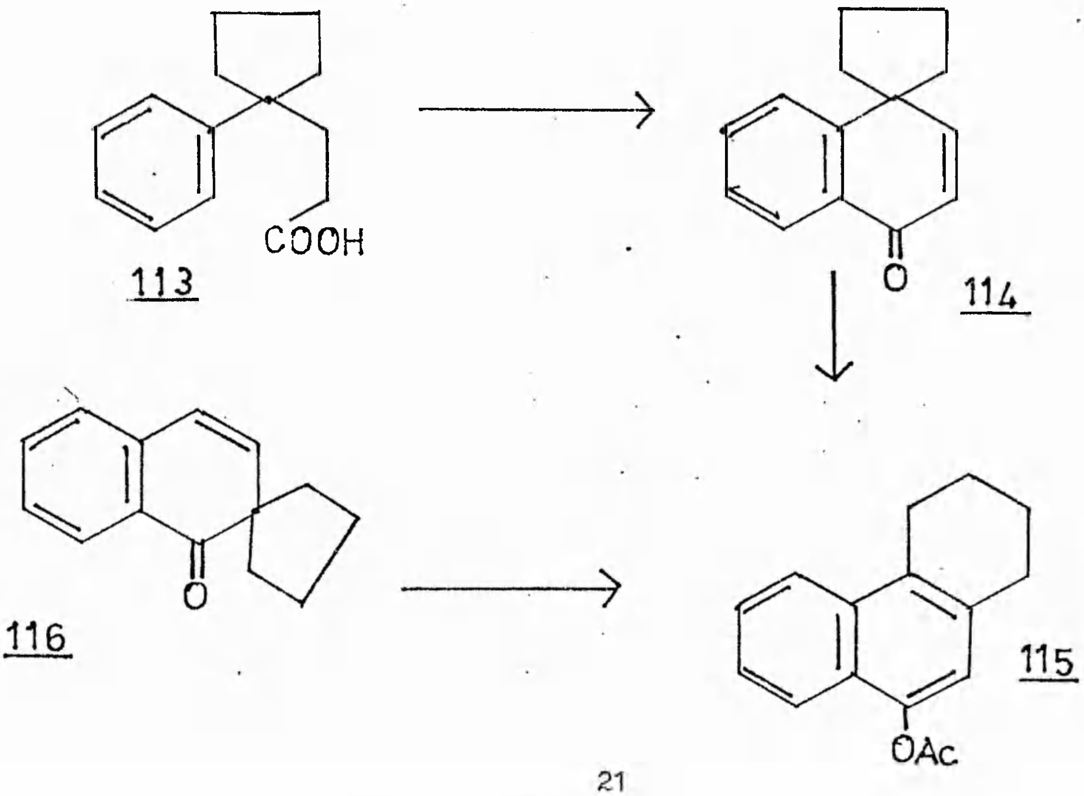
112



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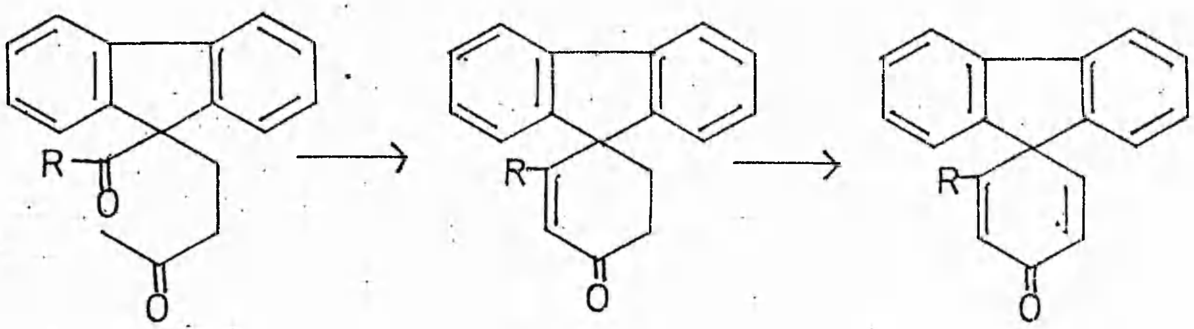
116

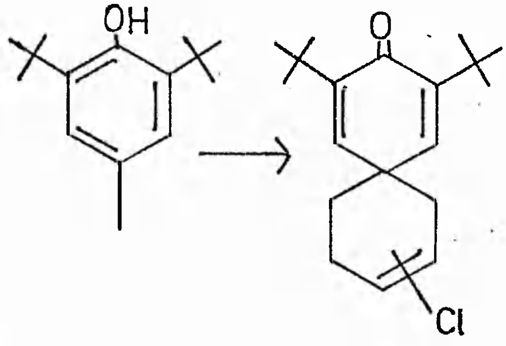
115

spirodienones had been synthesised by classical methods. The dienone 114 was prepared[77] by cyclisation of the carboxylic acid 113, bromination of the resulting tetralone and subsequent dehydrobromination with collidine. It was found to undergo a dienone-phenol rearrangement to give 115 in 95% yield on treatment with acetic anhydride-sulphuric acid. The analogous compound 116 was later prepared[78] by a similar route and under similar rearrangement conditions it gave a 91% yield of a product identical to the above (115). This result was later explained[38] by the mechanism discussed previously (see p. 7). In 1963 Plieninger et al[79] prepared 119 (R = H or $COOCH_3$) by condensation of 9-acylfluorenes with methylvinylketone to give 117, which was cyclised with piperidine in acetic acid to 118 and final selenium dioxide oxidation yielded 119. Dienone-phenol rearrangement of 119 (R = $COOCH_3$) gave the expected 3-acetoxy-1-carboxymethyltriphenylene, while lithium aluminium hydride reduction produced the dienol which, on treatment with 20% sulphuric acid in ethanol, was converted to 1-carbomethoxy-triphenylene.

Oxidation[80] of 4-alkylphenols in the presence of chloroprene by lead dioxide in benzene gave the spirodienones 120 and 121 in 75 and 5% yields; the position of the chlorine atoms on the lower ring was not determined. The proposed[80] mechanism involved attack of the intermediate hydroxy-di-t-butylbenzyl radical on chloroprene to form an allylic radical, which then cyclised to a spiro-radical, which was finally oxidised to the spirodienone (Scheme I). In an investigation[58] of p-quinone methides, it was found that they



117 118 119



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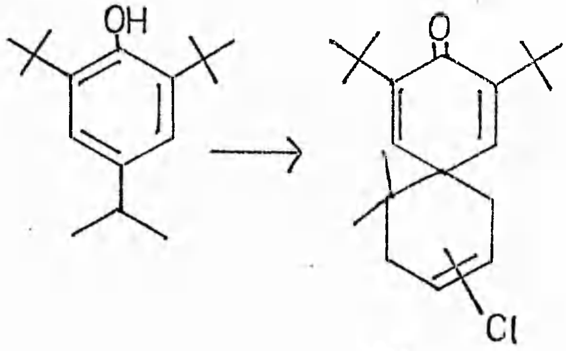

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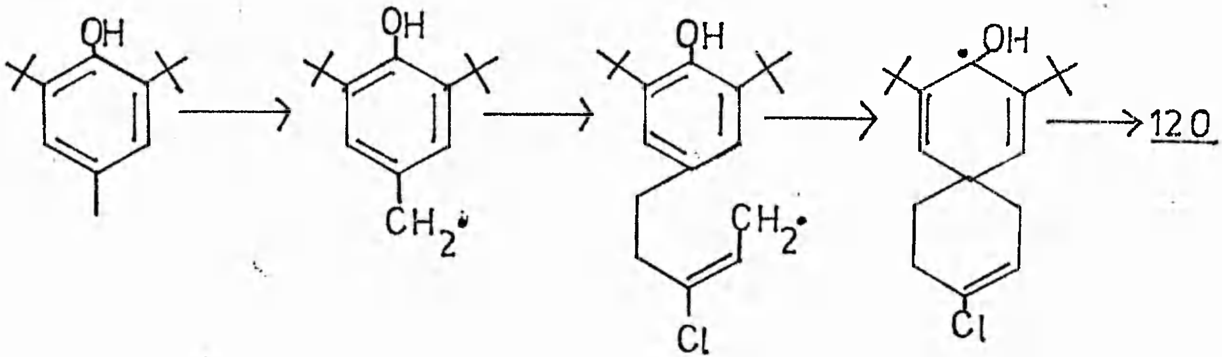
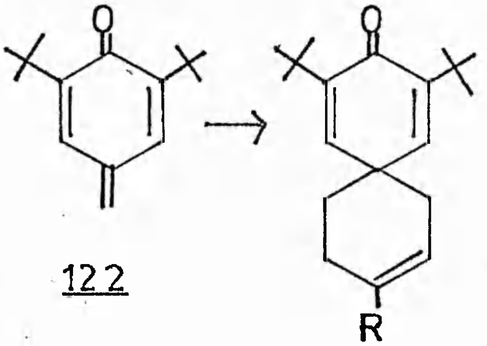
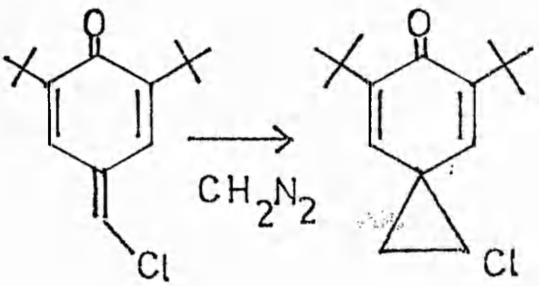


Scheme I



122 123



124 12.5

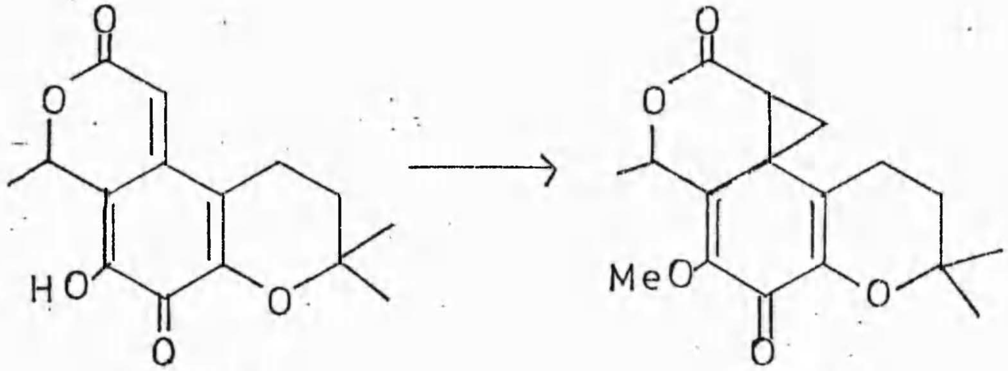
underwent Diels-Alder addition to butadienes to give spirodienones. By the addition of di-t-butylquinone methide 122 to butadiene, isoprene and chloroprene, were prepared 123 (R = H, Me and Cl); the substituent R was assigned to the 9-position rather than the 8 by analogy with known[81] reactions with 2-substituted butadienes. The formation[80] of 120 and 121 was explained by a similar mechanism, taking into account the recorded[82] formation of p-quinone methides by lead dioxide oxidation of 2,6-di-t-butyl-4-methylphenol.

Russian workers[83] reported a reaction of the chloroquinone methide 124 with diazomethane to form the spirodienone 125 in good yield. Similarly, Barton and Hendrickson[84] in their study of the constitution of fuscin 126, (a mould metabolite having a quinone methide structure), prepared a spirodienone 127, methylhomofuscin by treatment of 126 with diazomethane. The formation of a spirodienone 129 by dimerisation of the trimethyl-1,2-benzoquinone 128 in chloroform at room temperature has been reported.[85] The product underwent a dienone-phenol rearrangement to give 130.

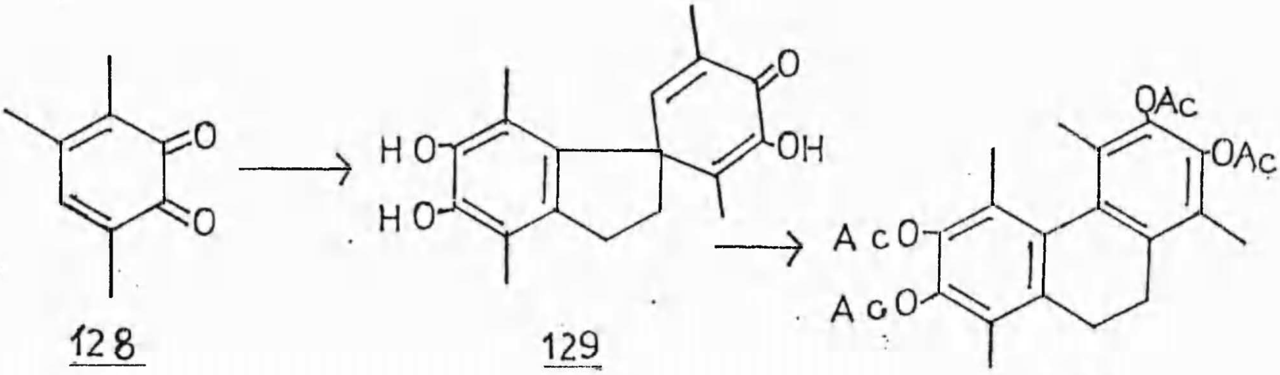
1.4 Heterocyclic Spirodienones

1.4.1 By Phenol Oxidation

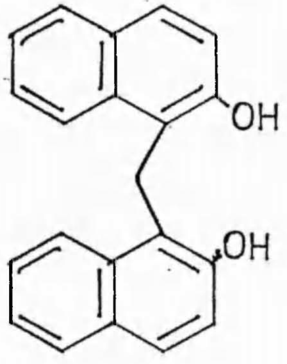
In 1892 Abel[86] oxidised 2,2'-dihydroxydinaphthylmethane 131 with basic hypohalite solution, and isolated a neutral product which he formulated as the peroxide 132. An alternative spirodienone structure 133 was later proposed[87] and this structure was finally confirmed[88] in 1931 by a second synthesis. Subsequently[89] the dihydroxydinaphthylethanes 134 (R = H or Br)



126 127



128 129 130



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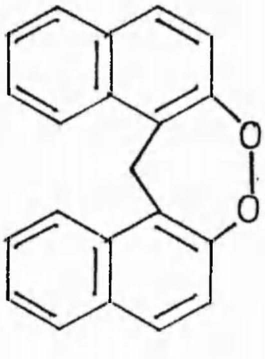

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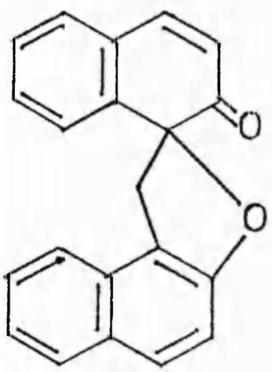

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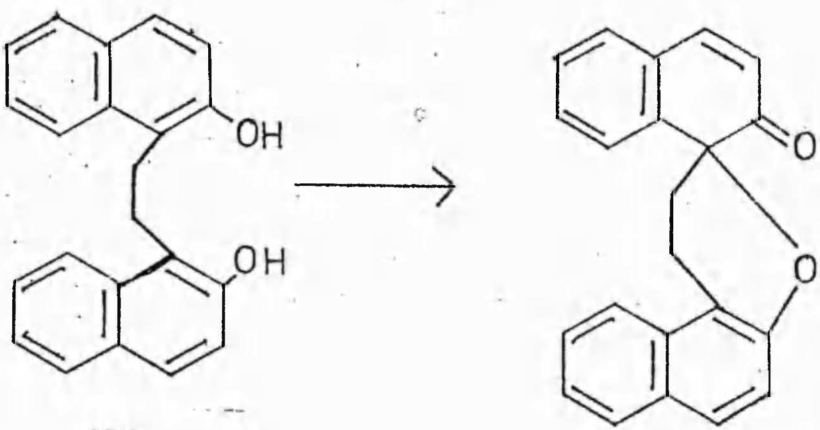

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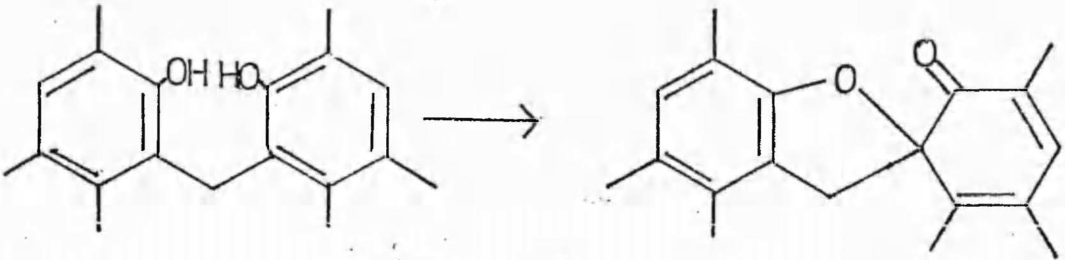

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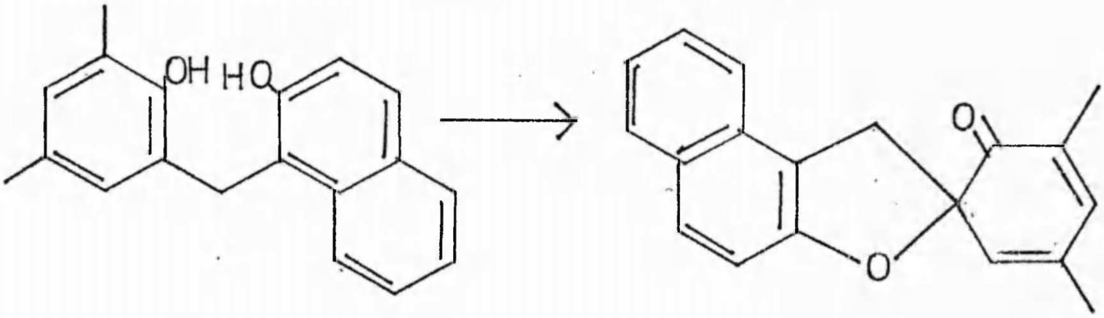

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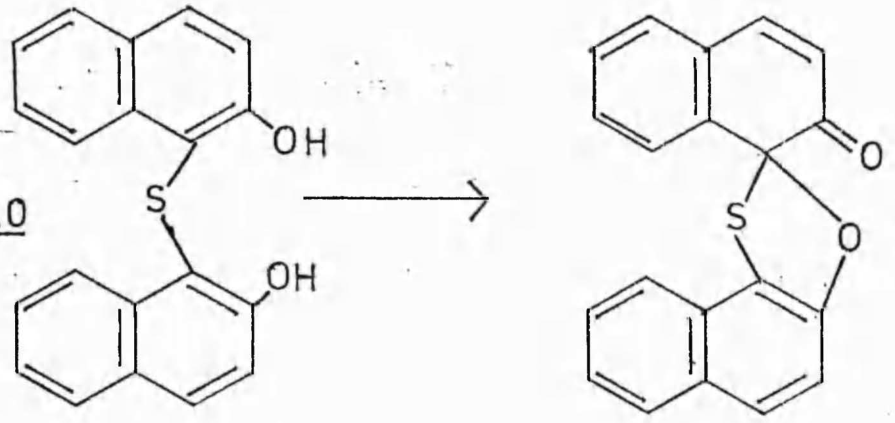
139

were oxidised to the corresponding dienones 135 with alkaline ferricyanide; while treatment of the diphenylmethane[88] 136 and the phenylnaphthylmethane[90] 138 with alkaline hypochlorite gave the corresponding dienones 137 and 139 in good yield.

The oxidation product obtained by treatment[92] of 2-naphthol-1-sulphide 140 with alkaline ferricyanide had erroneous structures proposed for it[93,94] before it was found[91] to be the spirodienone 141. Similarly the oxidation product[93] of 2-naphthol-1-sulphone 142 was shown[95] to be the spirodienone 143. McClement and Smiles[96] later prepared a series of spirodienones 144 (R^1, R^2, R^3 and R^4 = H, Me or Cl) by alkaline ferricyanide oxidation of the corresponding 2-hydroxyphenyl sulphides in an investigation of what was later to become the well-known Smiles rearrangement.

The thermal disproportionation[89] of "dehydro-1-methyl-2-naphthol" 145 in refluxing xylene or styrene produced the dienone 147, a reaction which was suggested[97] to proceed *via* the coupling of two molecules of the quinone methide 146. The alkaline ferricyanide oxidation of 2,2'-dihydroxydiphenylethanes gave[98] spirodienones 148 (R = H or Br) and a similar dienone 150 arose from coupling of two molecules of the bromide 149. The spirodienone 153 was prepared[99] from the diphenylmethane 151 in 80% yield by oxidation with the stable free radical, 2,4-di-*t*-butyl-6-phenylphenoxyl 152. This work was later extended[100] to the preparation of spirodienones 154 and 155 (R = H or Me, X = CH_2, CMe_2 and $CH_2 \cdot CH_2$) in 60-90% yields by oxidation of the appropriate diphenols with alkaline ferricyanide or the stable



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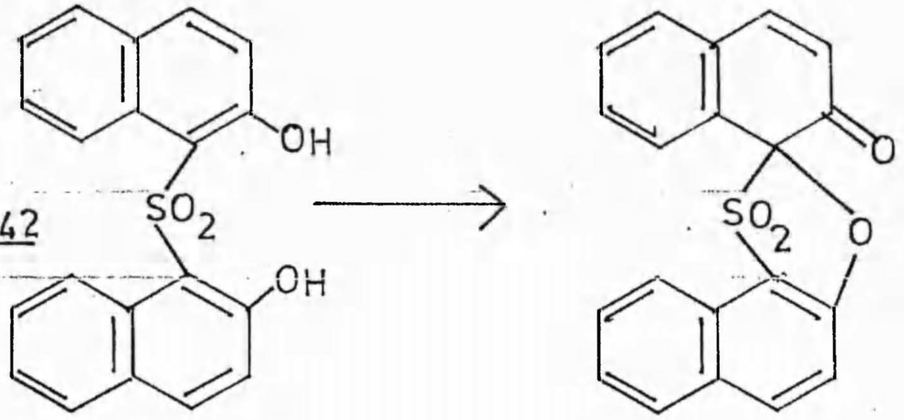

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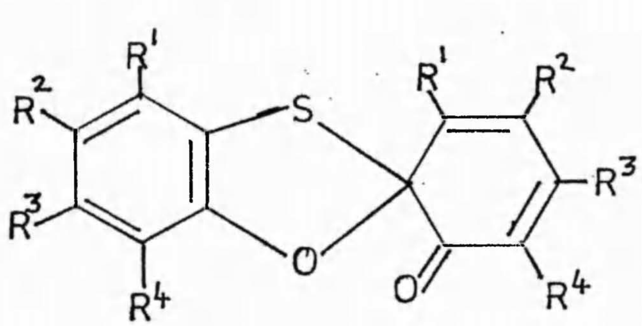

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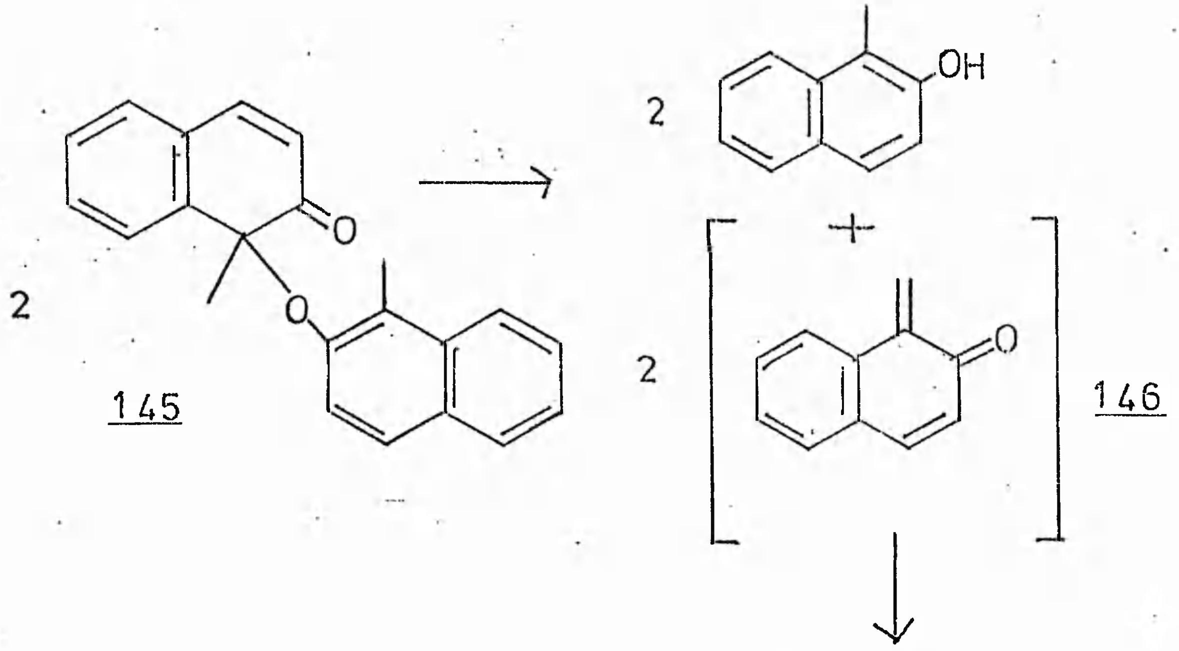

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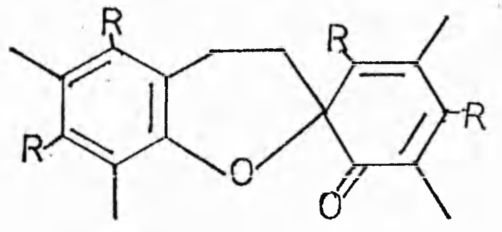

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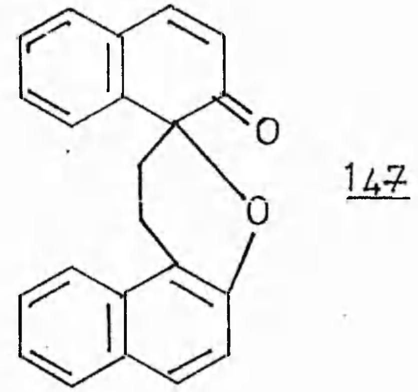

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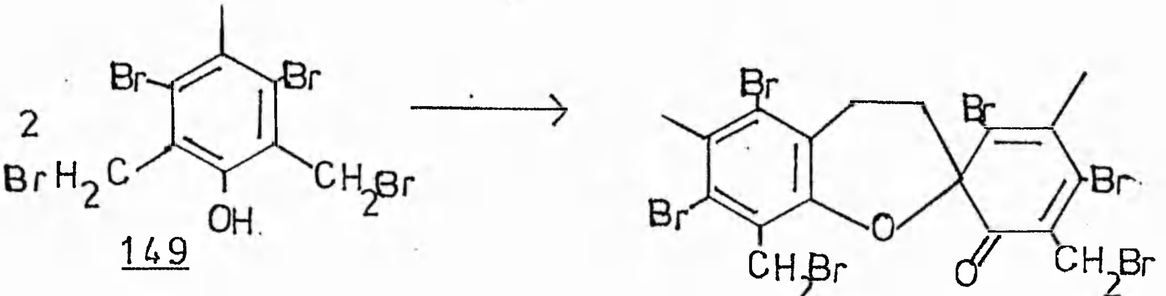

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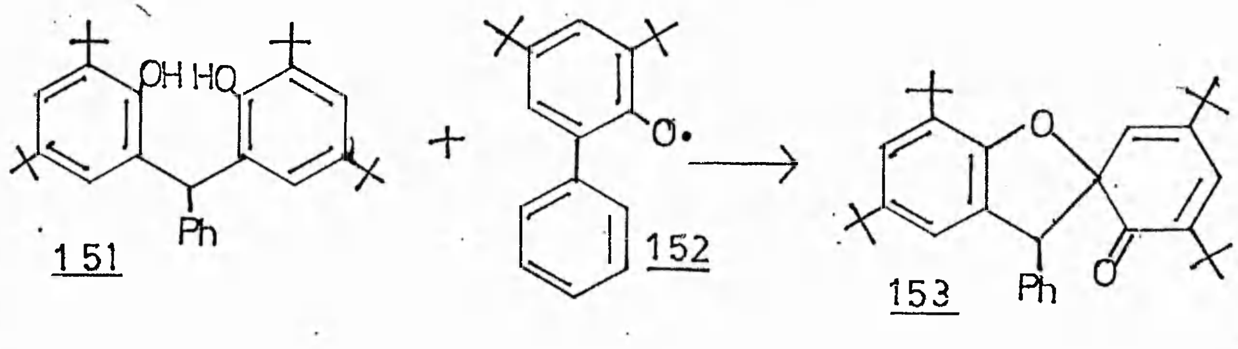

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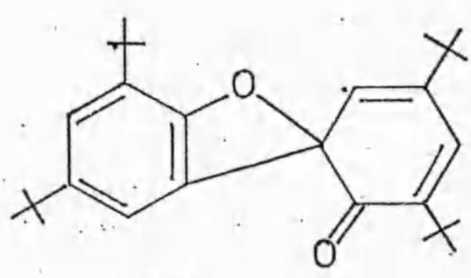
152

153

free radical, 2,6 di-t-butyl-4-cyanophenoxyl.

A similar spirodienone 156 had been isolated[101] from the alkaline ferricyanide oxidation of 2-*t*-butyl-4-methoxyphenol, but was wrongly formulated as the peroxide 157. The correct structure[102] was confirmed by Hewgill and his co-workers in one[103] of a series of papers on oxidation of alkoxyphenols; it was found that the dienone rearranged on treatment with acid to the hemiacetal 158. These workers had earlier prepared[104] the dioxepin spirodienone 159 by rearrangement of a blue solid obtained by oxidation of 3-*t*-butyl-4-methoxyphenol with ferricyanide, lead dioxide or silver oxide. It was later found[105] that similar treatment produced an analogous product 160 in 70% yield from 4-*t*-butyl-2-methoxyphenol. Extension of this reaction[106] led to the preparation of a series of dioxepin spirodienones 161 (R or R′ = Me, Et, *iso*Pr and Ph) in 8-20% yields by the oxidation of 2- or 3-substituted-4-methoxyphenols. The authors proposed that formation of dioxepin spirodienones is a general reaction of 2,4-substituted phenols and in particular when one of the substituents is alkyl and the other alkoxy, except for a 2-*tert*-alkyl-4-alkoxyphenol which would give a benzoxet ? similar to 156. They reported[107] that oxidation of a mixture of 2- and 3-*t*-butyl-4-methoxyphenols gave a mixed product 162 together with 159. This product 162 was also produced in 50% yield by alkaline ferricyanide or silver oxide oxidation of a mixture of 4,4′-di-*t*-butyl-2,2′-dihydroxy-5,5′-dimethoxy-biphenyl



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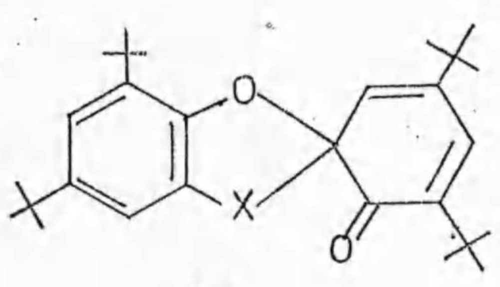

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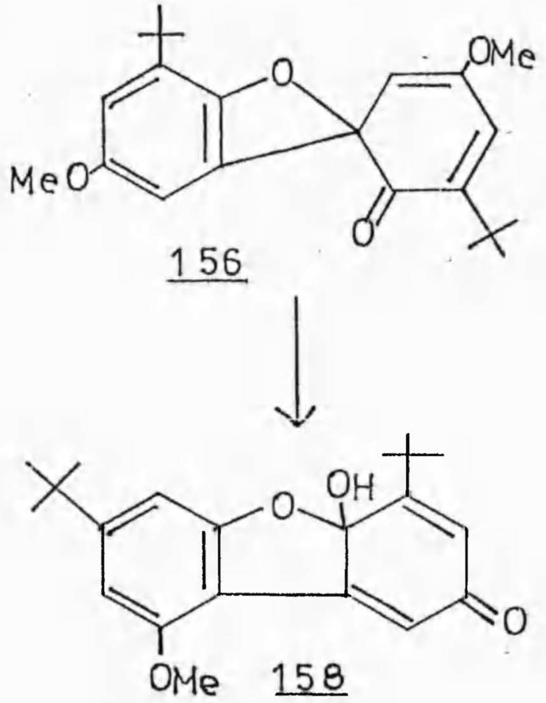

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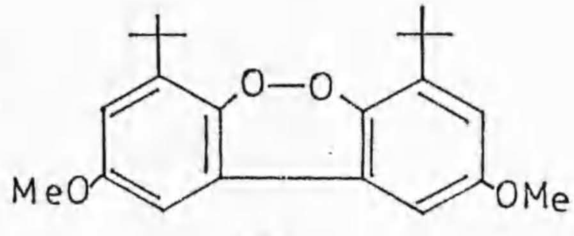

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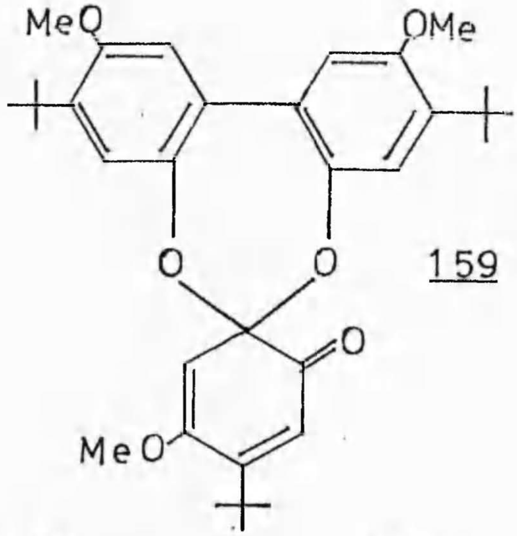

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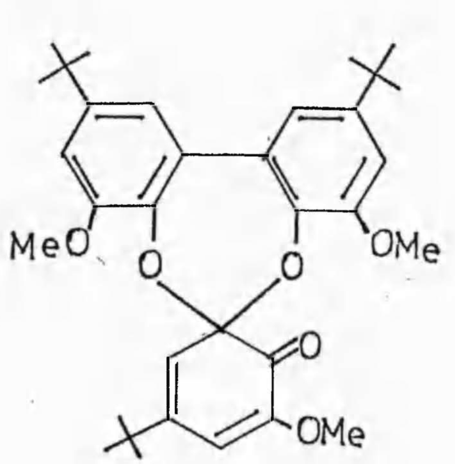

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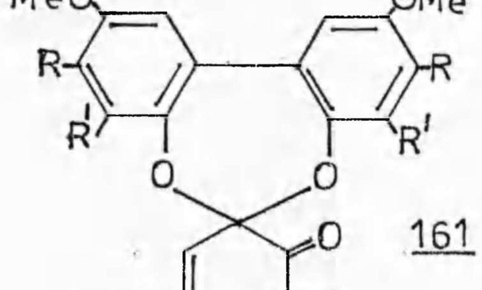


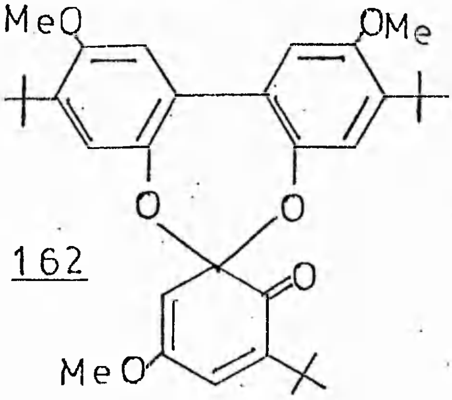
161

and 2-t-butyl-4-methoxyphenol. Another product 163 was isolated and a later report[108] detailed the oxidation of a mixture of this and 3-t-butyl-4-methoxyphenol with silver oxide to give the mixed spirodienone 162 in 90% yield. The same authors found[109] that reaction of substituted bromophenols with base in DMF gave similar products; 2-bromo-4-t-butyl-6-methylphenol gave 164 in 95% yield, 2-bromo-5-t-butyl-4-methoxyphenol gave 159, while 2-bromo-4,6,di-t-butylphenol yielded 165 in agreement with their earlier suggestion. The proposed mechanism involves electron transfer from the phenoxide ion to the keto-tautomer of the phenol which subsequently loses bromide, with the resulting pair of radicals entering a chain reaction. Becker[110] produced a dioxepin spiro-dienone 166 in 54% yield by ferricyanide oxidation of 2,4-diphenylphenol.

Spirodienones 168 have been prepared[111] by oxidation of the substituted salicyl alcohols 167 (R^1 and R^2 = Me, t-Bu, Ph, hydroxymethyl, cyclohexyl and I) with sodium periodate in methanol. Yields were greater than 70% and the products underwent a photolytic rearrangement to salicylaldehydes.

The synthesis of heterocyclic-1-spiro-2,5 dienones is a more recent development. Interest was aroused because of the presence of this structure in the fungal metabolites, (see later).

The dienone 170 has been prepared[62] in 80% yield by oxidation of the phenolic carbonate 169 with alkaline ferricyanide while the carbocyclic dienone 63 was converted[61] into the peroxide 171 by reacting a solution with oxygen. Oxidation of an ethereal solution



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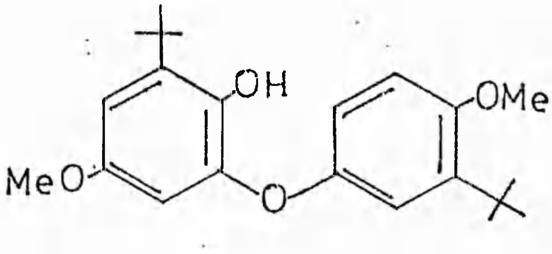

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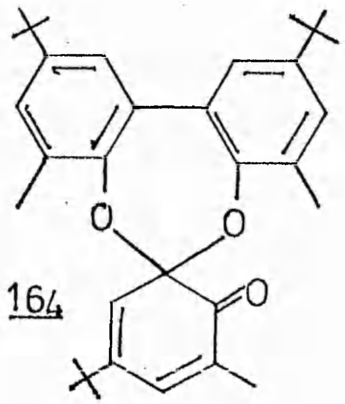

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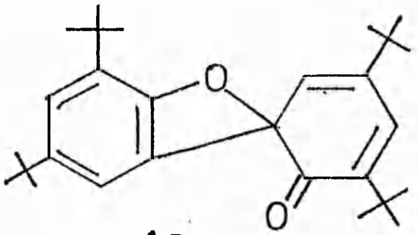

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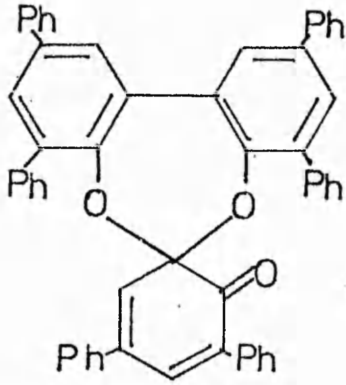

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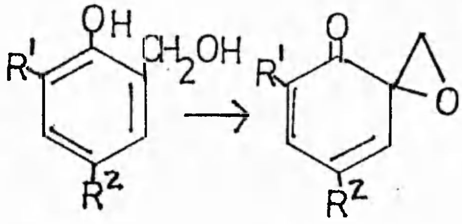

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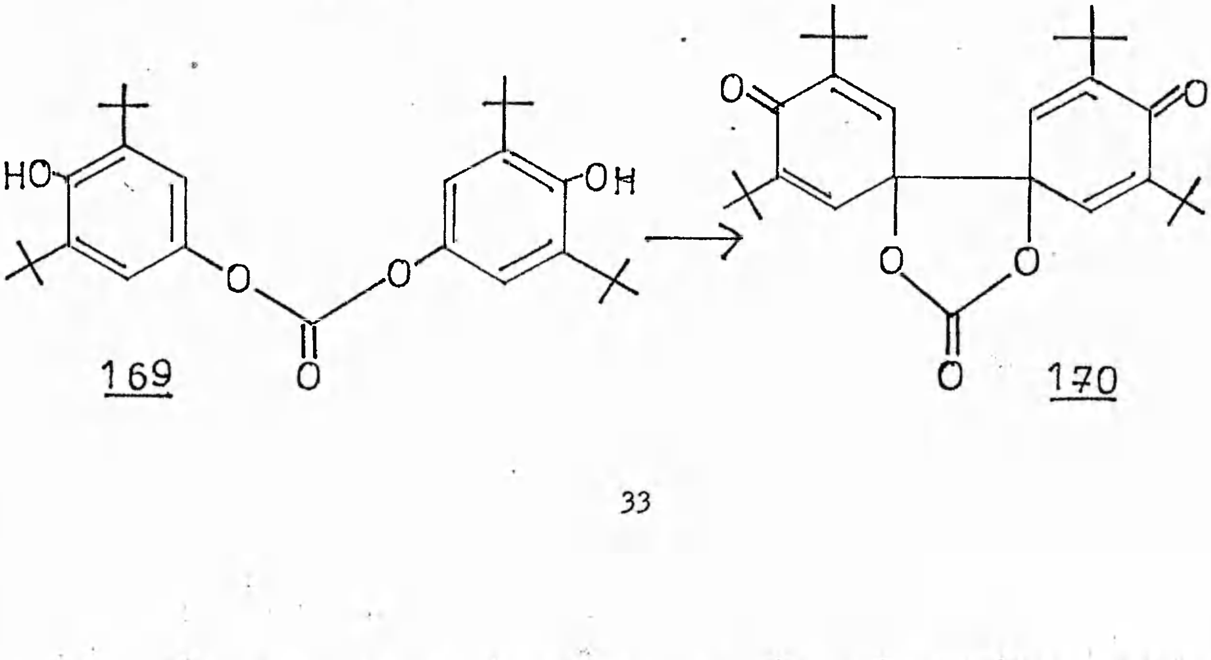

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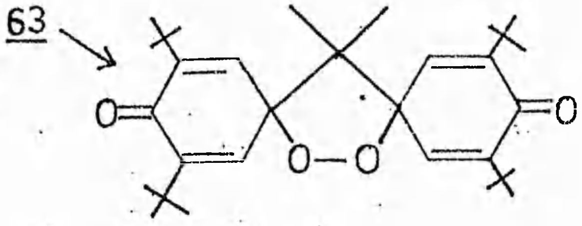
of 2,4'-dihydroxydiphenylethane with ferricyanide gave[112] the spirodienone 172 in 11% yield. Later[113] the dienone 173 was similarly prepared from 2,4'-dihydroxydiphenylmethane in 2% yield and dienone-phenol rearrangements carried out on 172 and 173 yielding 174 and 175 respectively, showing that carbon bond migration had taken place exclusively. Compound 176 was prepared[114] similarly from the diphenylethane in 1% yield. Several spirodienones have been obtained[115,116] by oxidation of dihydroxydiaryl ethers with manganese dioxide or DDQ. Thus the diphenols 177, 179 (R = H or Me), 181 and 183 gave the corresponding dienones 178, 180, 182 and 184. Yields were generally low (1-15%) except when stabilising factors were present in the product e.g. 180 (R = H), 100% yield. None of these compounds underwent dienone-phenol rearrangements.

Phenolic carboxylic acids have been converted to 2,5-dienones. Oxidation[117] of 185 by ammonium ceric sulphate or lead dioxide produced the spirolactone 186 in moderate yields. On treatment with boron trifluoride in acetic anhydride 186 rearranged to the xanthone 187. A similar preparation was the oxidation[118] of 4'-hydroxybiphenyl-2-carboxylic acid to the dienone 188 by manganese dioxide in ether in 25% yield. Compound 188 had previously been prepared[119] by an acid catalysed Bamberger rearrangement of the biphenylhydroxylamine 189. The dienone 188 underwent rearrangement in hot 20% sulphuric acid to the benzocoumarin 190, a migration of the oxygen bond, while hot 10% sodium hydroxide gave the isomeric



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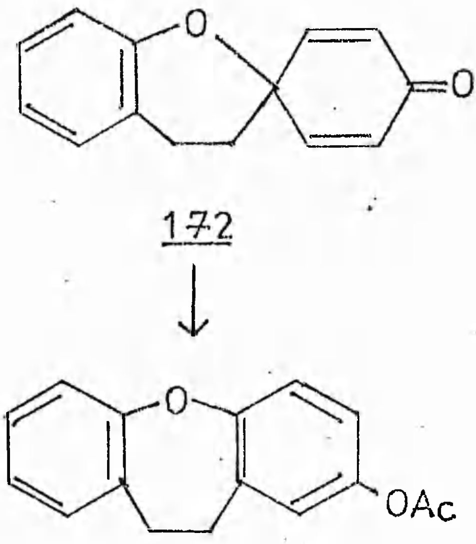

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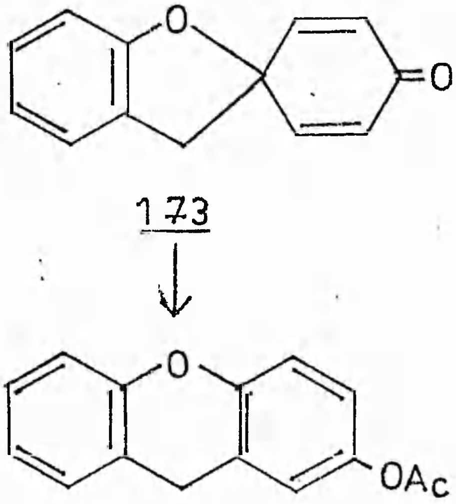

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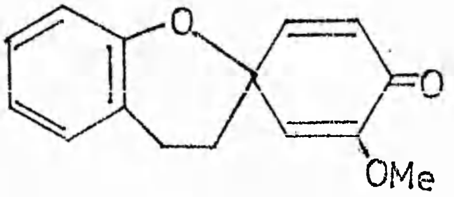

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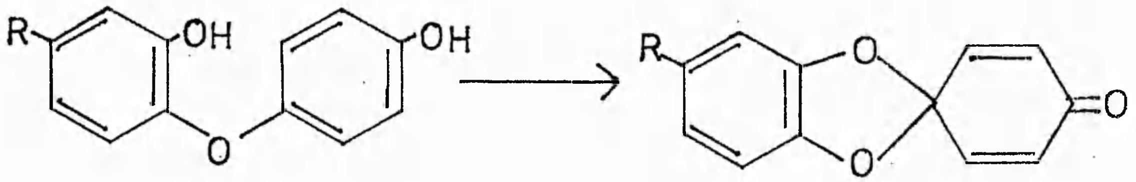

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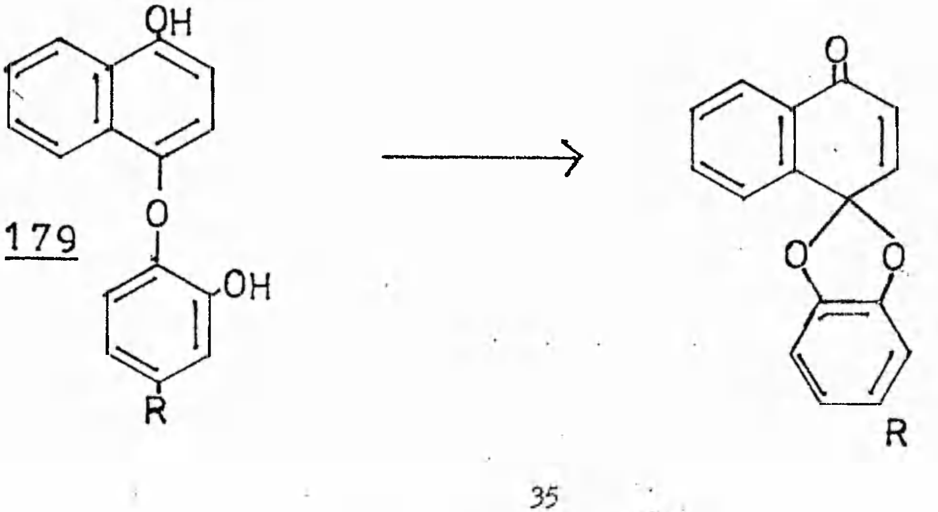

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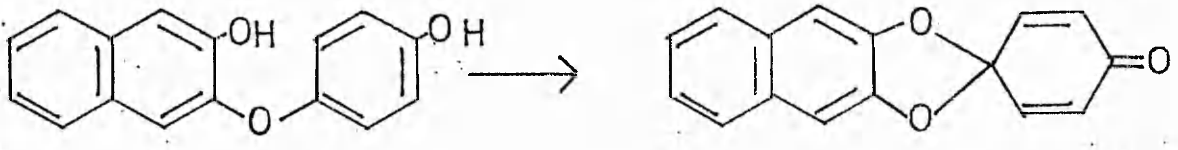

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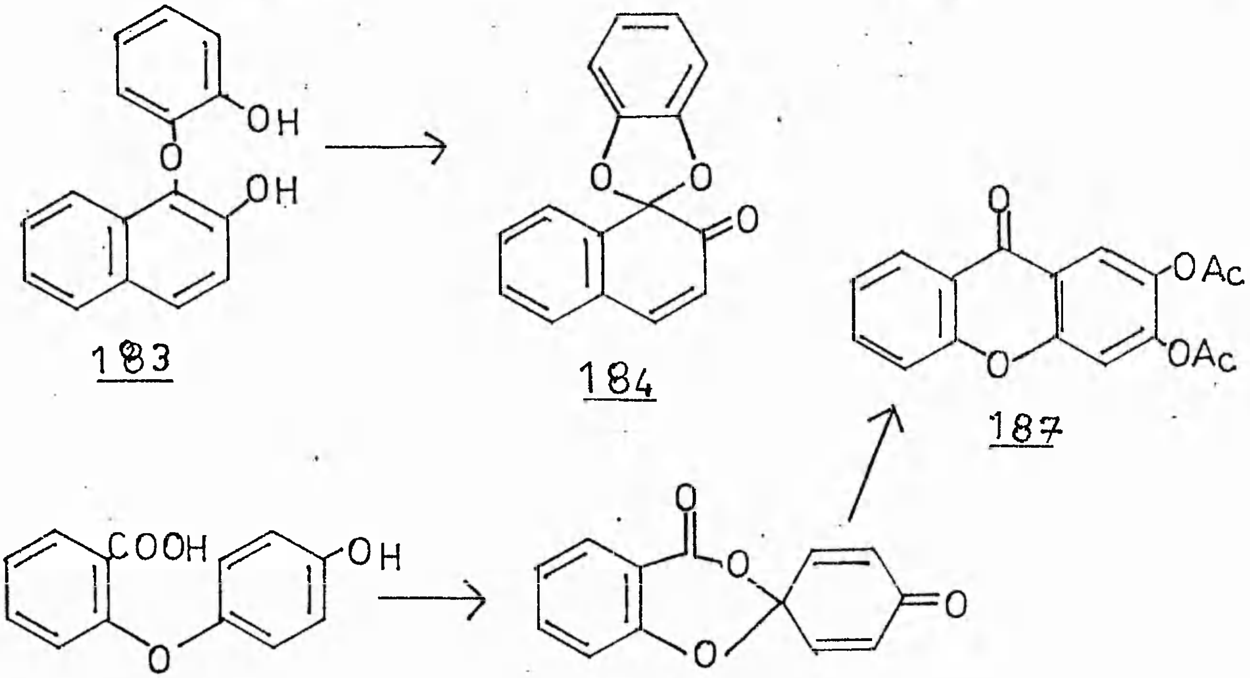

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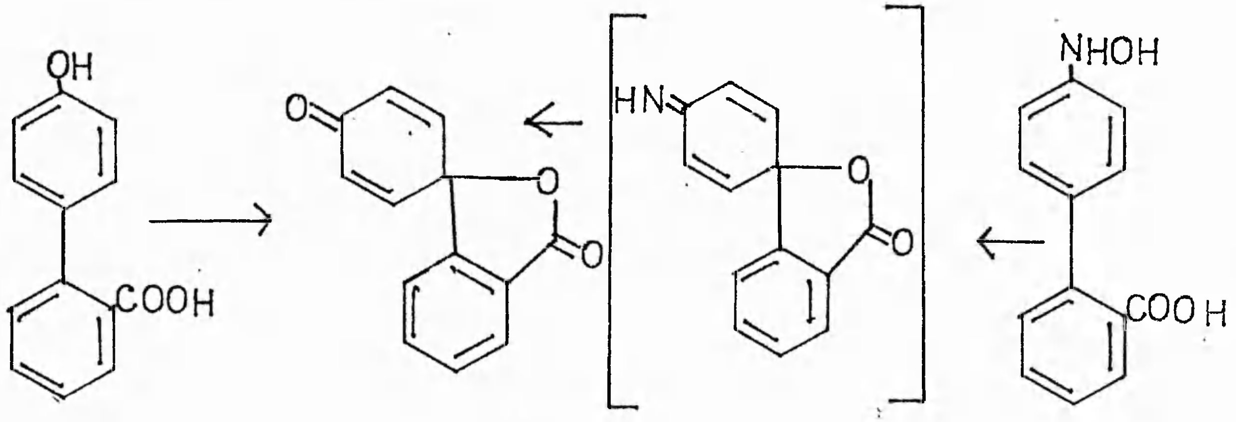

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compound 191.

Hassall et al[120] reported on the oxidation of dihydroxybenzophenones, in connection with the synthesis of griseofulvin. The spirodienones 192 (R = H or Me) were obtained by lead dioxide oxidation of the corresponding benzophenones in 8 and 70% yields respectively. It was found, however, that 2,4′-dihydroxybenzophenone was unaffected by lead dioxide or alkaline ferricyanide and this led to the proposal that substitution in the 6,2′ and 6′ positions assisted the oxidation by disturbing the co-planarity of the two rings. Treatment of the dienone 192 with acid produced the diphenyl ether 193.

The search[23] for phenoxylium ion intermediates has yielded new spirodienones. The carboxydiphenylethers 194 (R^1 = tBu, R^2 = H and R^1 = R^2 = tBu) gave the dienones 195 in 60% yield on oxidation with lead dioxide in ether. The related ester 196 was similarly converted quantitatively to the spirobenzoxet 197; this reaction conforms to the principle proposed by Hewgill.[106] (see p. 30). The diphenylmethane derivatives 198 and 200 were oxidised to the dienones 199 and 201 in 10 and 50% yields by lead dioxide and lead tetra-acetate in benzene respectively. The dienone-phenol rearrangement of 201 with boron trifluoride in acetic anhydride gave 202, an example of oxygen bond migration; the decision between the two isomeric dibenzoxepins, however, rests only on NMR data.

The oxidation of the aryloxyacetic acid 203 with persulphate has been reported[121] to give a dimeric compound which was

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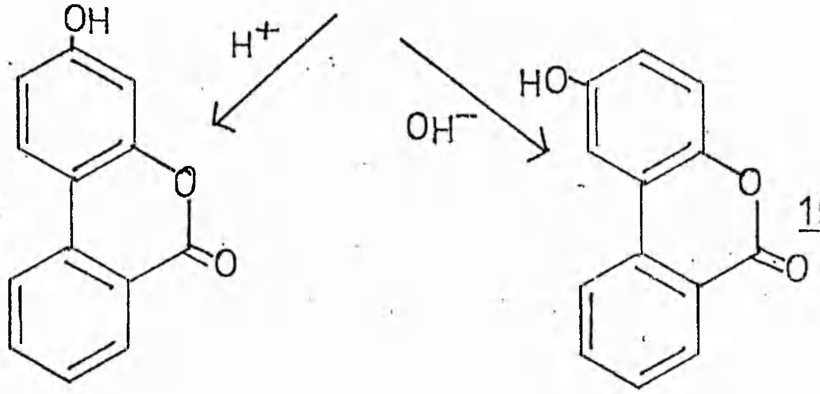

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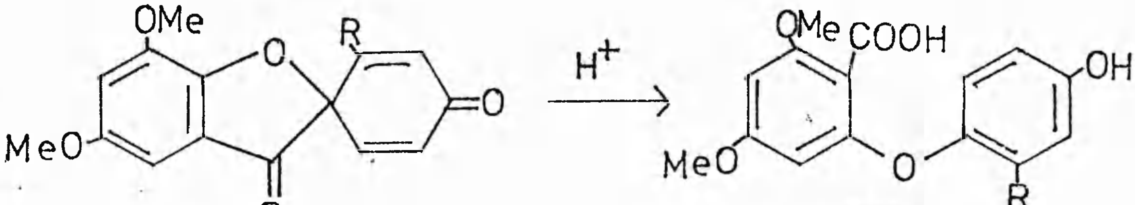

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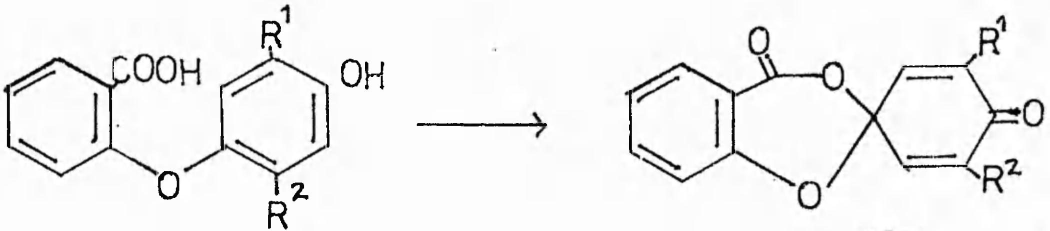

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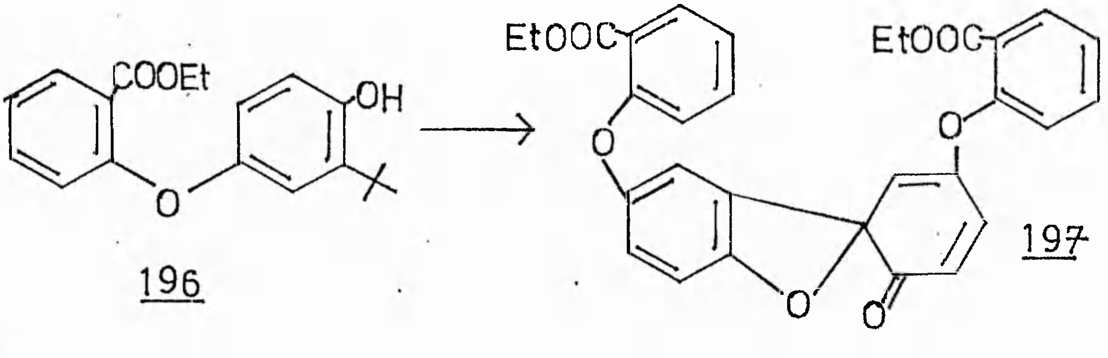

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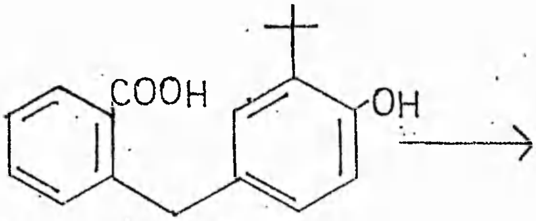
formulated as a Diels-Alder adduct of two molecules of the spirodienone 204. The dimer could, on heating, be equilibrated with three isomeric dimers and the monomer could be trapped by Diels-Alder addition to maleic anhydride.

The observation[122] that the peptides oxytocin and vasopressin were cleaved at tyrosyl residues by bromine water led to further investigations.[123,124] Treatment of 3-(4-hydroxyphenyl)-propionic acid (phloretic acid) with N-bromosuccinimide gave the spirolactone 205 (R = Br) in 86% yield. Related dienones 206 (R = Br; R' = Me, Ph or $PhCH_2O$) were prepared by similar treatment of N-acyltyrosines. Confirmatory results were published simultaneously.[125] Electrolysis of phloretic acid at a platinum anode[126] gave 205 (R = H) in 20% yield and similar treatment of N-carbomethoxytyrosine gave 206 (R = H, R' = OMe). Electrolysis of 4-hydroxy-cis-cinnamic acid yielded the unsaturated spirolactone 207. Other workers[127] prepared 205 (R = H) by electrolysis of N-[3-(4-hydroxyphenyl)propionyl] glycine. Oxidation[128] of phloretic acid by acetic acid-hydrogen peroxide, peracetic acid and lead tetra-acetate gave low yields of 205 (R = H). Japanese workers[129] produced spirolactones 205 (R = I, Br or Cl) by photolysis of the dihalophloretic acids in the presence of a sensitizer, erythrosin, and oxygen, and also by the oxidation of the dihalophloretic acids by N-bromosuccinimide and by a mixture of hypochlorite and hydrogen peroxide in aqueous methanol. Other workers[130] produced similar di-iodospirolactones by the action of N-iodosuccinimide on phloretic acid and related



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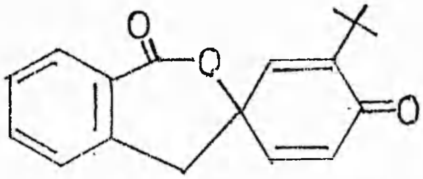

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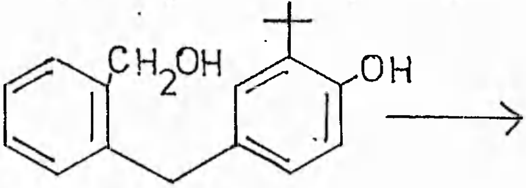

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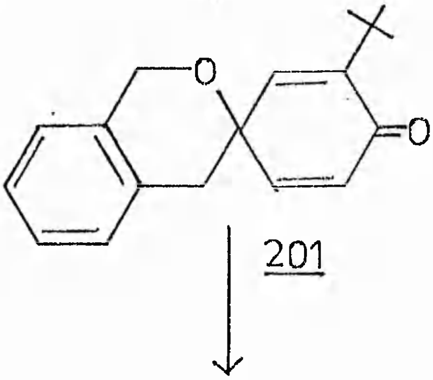

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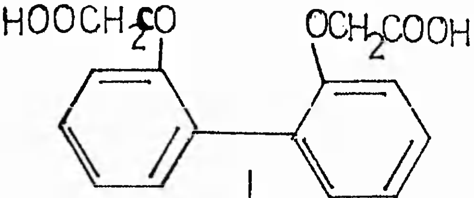

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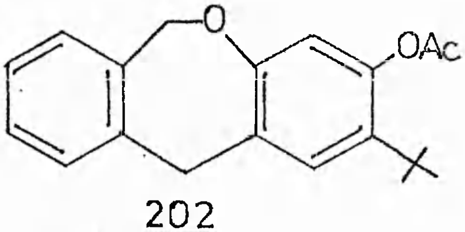

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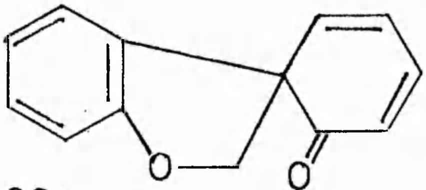

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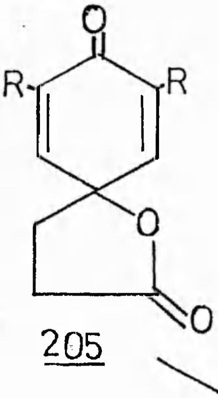

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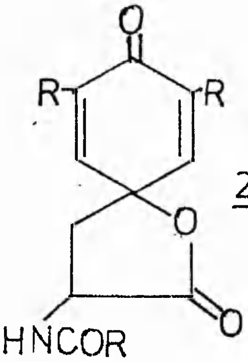

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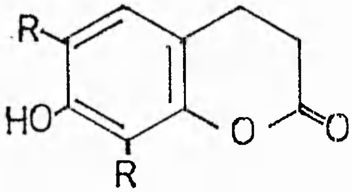

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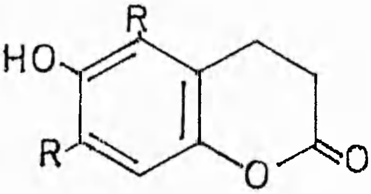

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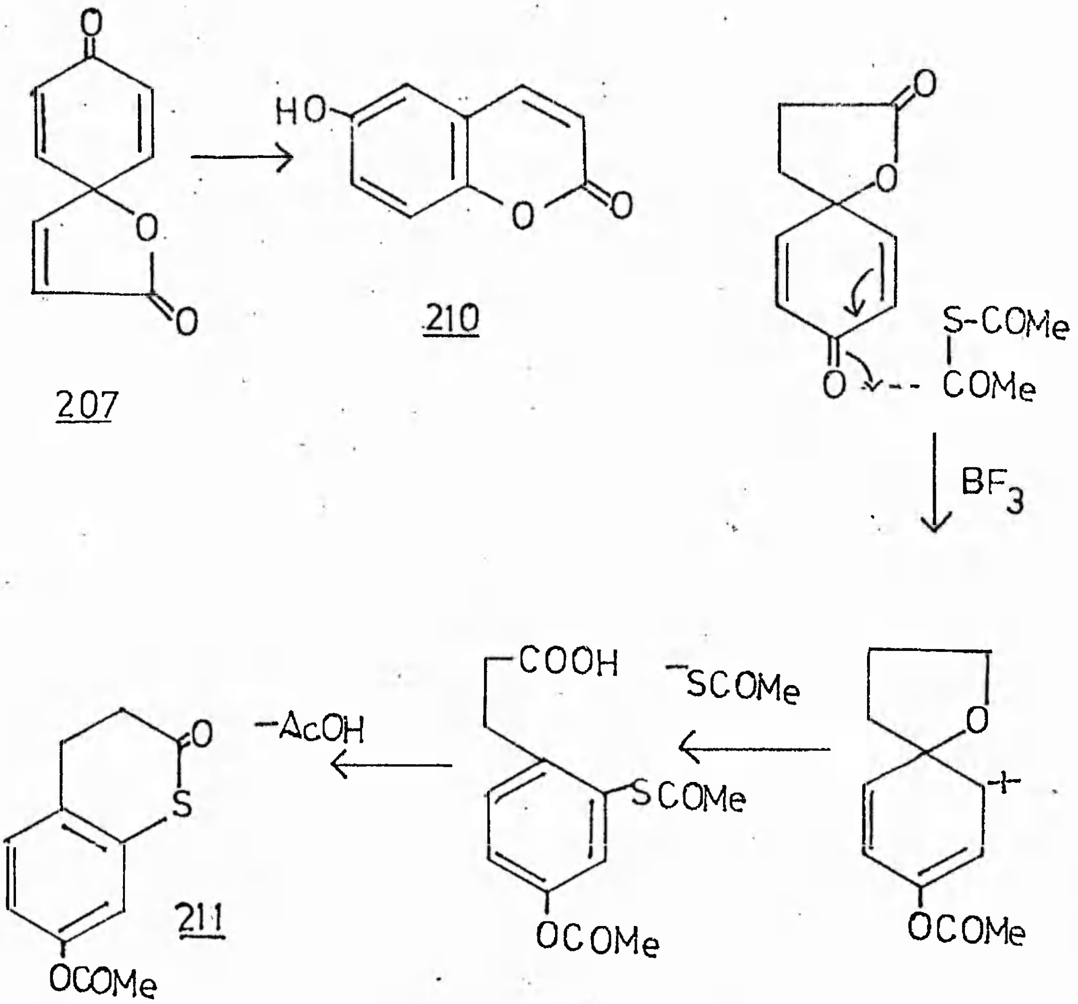
amides.

The acid catalysed dienone-phenol rearrangement of these spirolactones is of interest as a proposed step in the biogenesis of coumarins. Treatment[124] of 205 (R = Br) with 2M sulphuric acid for 2 hours produced oxygen migration to give the dihydrocoumarin 208 (R = Br). The same product was obtained[126] using 4.5M sulphuric acid in acetonitrile. Further investigation[126] showed that the type of migration occurring depended on the conditions; 205 (R = H) gave 95% of 6-hydroxydihydrocoumarin 209, (carbon migration) on treatment with 0.5M sulphuric acid while acetic acid containing sulphuric acid gave the 7-isomer, (oxygen migration) in 90% yield. The unsaturated spirolactone 207 rearranged to 6-hydroxycoumarin 210 on treatment with 3M sulphuric acid. Other workers[128] confirmed these findings and found rearrangement of 205 (R = H) occurred with oxygen migration on solution in acetic anhydride-sulphuric acid. When O^{18}- labelled acetic anhydride was used the resulting coumarin was also labelled at the ring oxygen. Rearrangement in diacetyl sulphide-boron trifluoride led to 7-acetoxy-3,4dihydrothiocoumarin 211, and a mechanism was proposed in which initial attack of an acetyl unit on the dienone carbonyl group led to attachment of the thioacetate group at the 2-position of the ring, followed by cyclisation to the thiocoumarin (Scheme II).

Electrolysis of the isochromanone 212 in acetonitrile gave[131] a 55% yield of the bicyclic spirolactone 213, which on treatment with methanolic hydrochloric acid lost formaldehyde to form the



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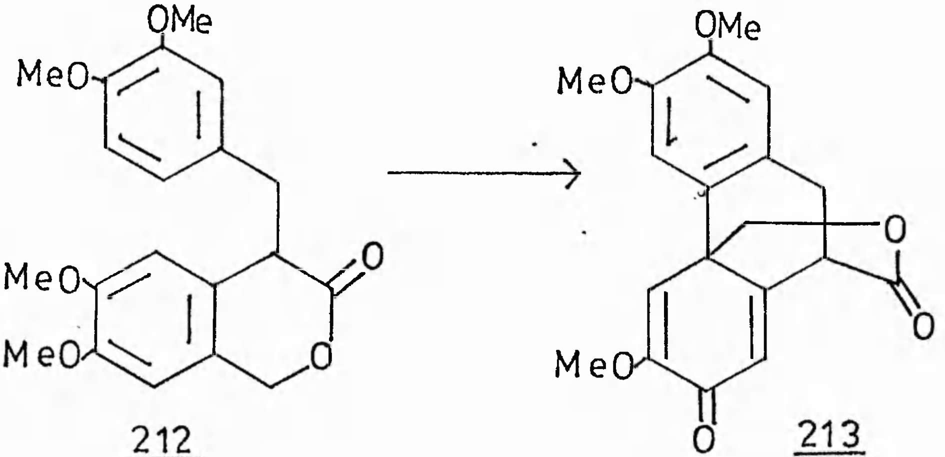
Scheme II



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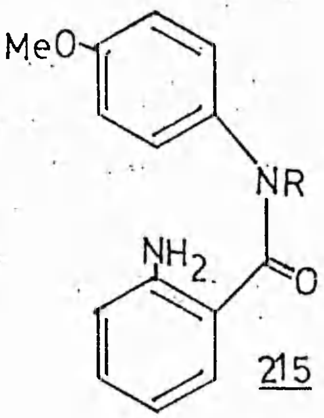
dihydrophenanthrene 214.

Nitrogen containing heterocyclic spirodienones are less common. Hey and his co-workers have made a study of spirodienone lactams. Compound 216 (R = Me or Et) was first isolated[132] from the decomposition of the diazobenzanilide 215, in 48% yield. The 2-methoxy isomer gave a low yield of the 2,4 dienone 217. It was later found[133] that the same compounds were formed by treatment of N-alkyl-2- or 3-bromo-4′-hydroxybenzanilides with potassamide in liquid ammonia; this is good evidence for participation of a benzyne intermediate. Compound 216 was also formed[134] in 84% yield by the pyrolysis of the dimer 218 (R = Me or Et), in the presence of iodine as an oxidant. The persulphate oxidation of biphenylcarboxamides gave spirolactams.[135] Compound 216 (R = Me) was formed from N-methylbiphenyl-2-carboxamide and also from the 4′-methoxy analogue. Oxidation of N-methyl-2′-methoxybiphenyl-2-carboxamide gave 217 (R = Me, 45% yield) and 219 in 25% yield. The dienol 220 was prepared[136] by photolysis of N-ethyl-2-bromo-4′-hydroxybenzanilide in aqueous alkali in the presence of sodium borohydride; on oxidation by manganese dioxide it gave the dienone 216 (R = Et, 59% yield). Similarly,[137] N-methyl-2-iodo-4′-methoxybenzanilide gave 216 (R = Me) on photolysis in oxygen-free benzene while the same product was obtained by similar treatment of N-methyl-2-iodobenzanilide in benzene saturated with oxygen. . Compounds 216 (R = H, Me or Ph) were obtained[138] by the oxidation of the appropriate biphenyl-2-carboxamides with t-butyl hypochlorite and iodine. Naphthalene



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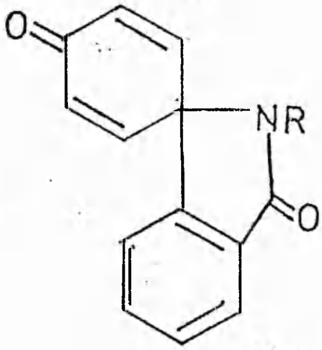

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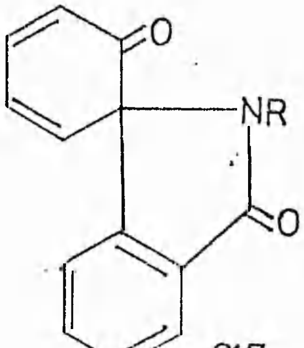

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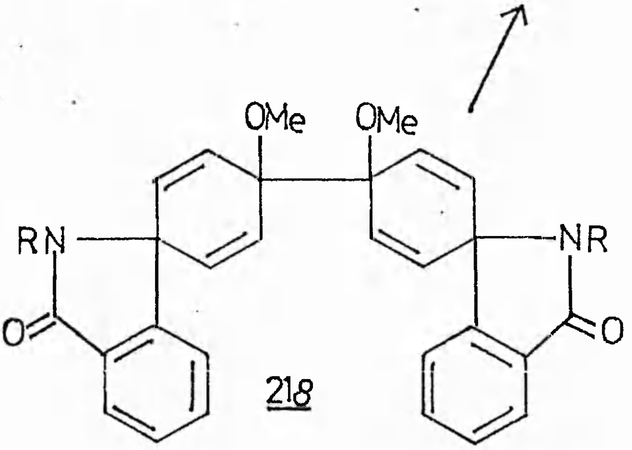

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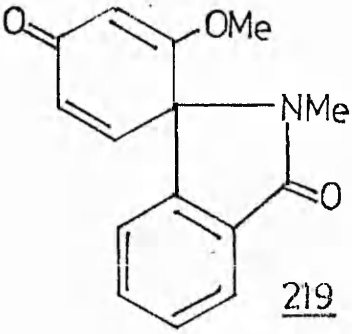

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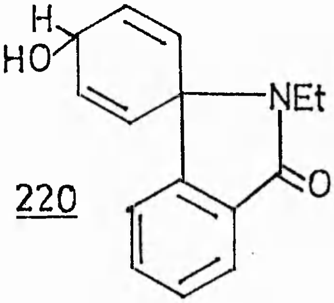

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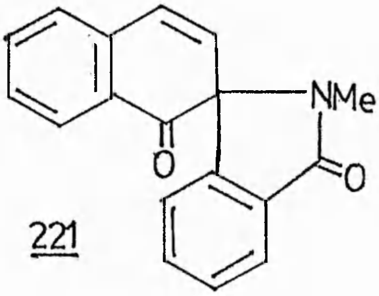

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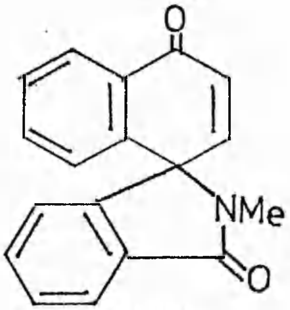

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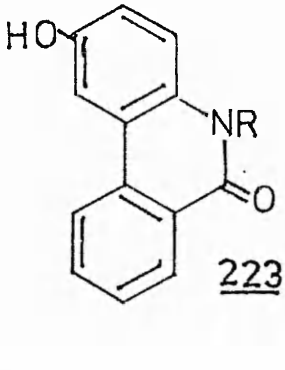

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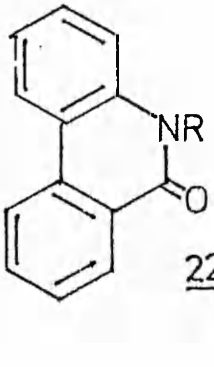

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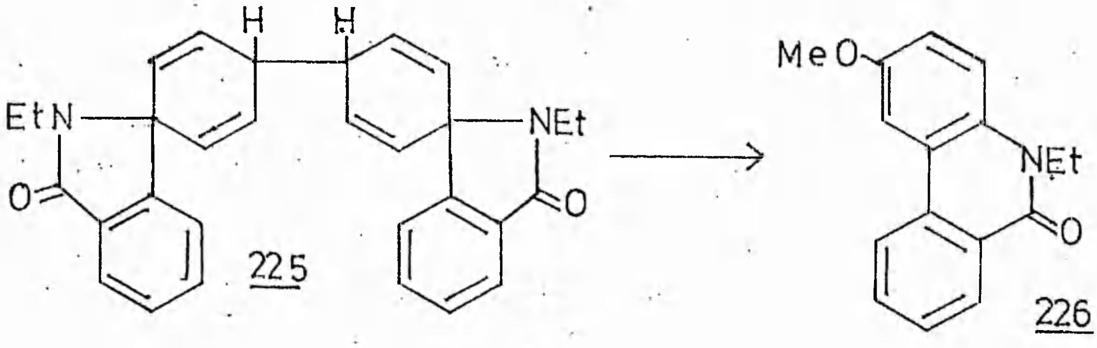
analogues 221 and 222 of these spirolactams have been prepared by decomposition of diazonium salts.[139,140]

Rearrangements of 216 have been reported;[132] on treatment with 100% phosphoric acid at 170° the phenanthridone 223 was formed, with carbon rather than nitrogen migration. The 3-acetoxyphenanthridone was also formed by heating 216 in acetic anhydride containing sulphuric acid at 107° for 2 hours. Reduction of 216 gave the dienol 220 which rearranged on heating with hydrochloric acid to form the phenanthridone 224. On treatment with base the dienol gave the 4′-hydroxybiphenyl-2-carboxamide.

Nitrogen bond migration, however, has been noted[139] in pyrolysis of the spirodienyl dimer 225 to yield 226, which is only one of the four possible isomeric products (2 by carbon and 2 by nitrogen migration). This rearrangement involves a spirodienyl radical as opposed to the dienone-phenol case which proceeds by a spirodienyl cation. Compound 216 is converted[135] to its isomer 217 on photolysis in oxygen free benzene and also to a mixture of two geometrical isomers 227 and 228 on irradiation in the presence of iodine.

The oxidation of phenolic oximes has been studied[141] in an investigation into the synthesis of aerothionin, a sponge metabolite. Oxidation of a series of 4-hydroxyarylpropan-2-one oximes 229 with manganese tris(acetylacetonate) in acetonitrile gave spiro-isoxazolines 230 (R^1 = H, t-Bu or Br; R^2 = H or Me; R^3 = H, Me or COOMe) in yields of 20-60%. This reagent did not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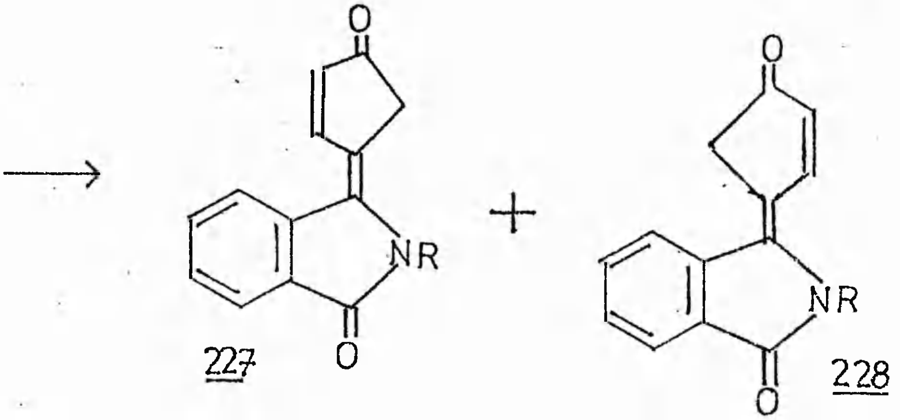
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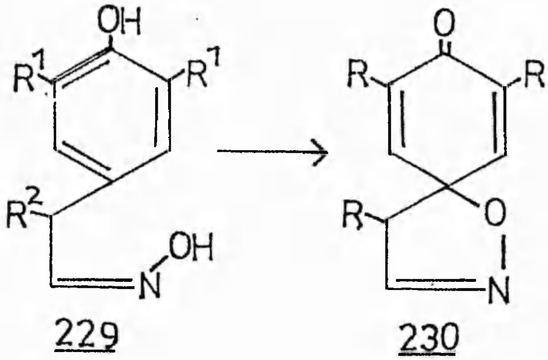
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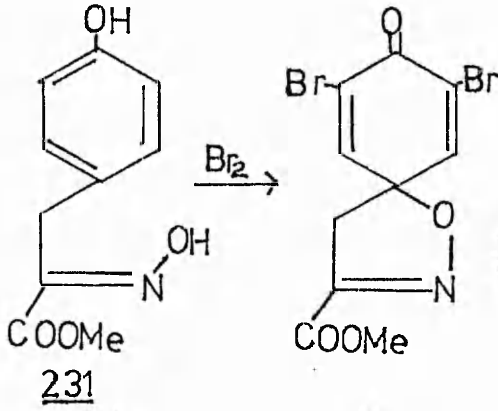

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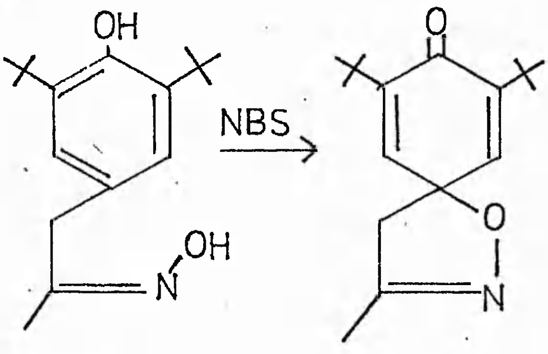

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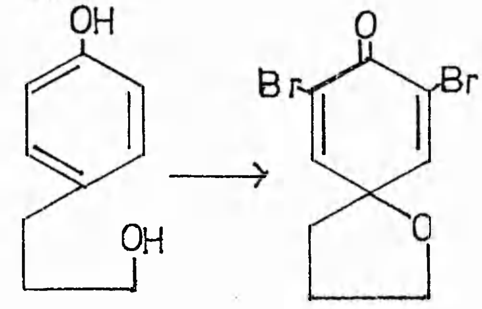
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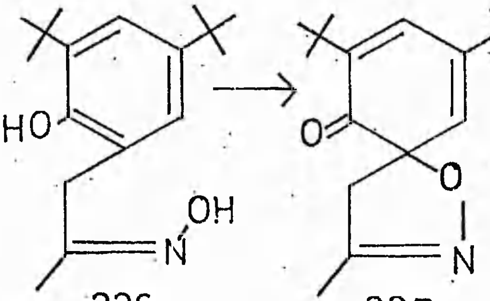
produce dienones from the 2-hydroxyaryloximes probably because the phenol and oxime groups were close enough to intereact with the manganese. The mechanism is considered unlikely to proceed through a bi-radical but rather by trapping, by the oxime, of an incipient phenoxium ion produced by heterolysis of the oxygen-manganese bond in the metal-phenol complex. Bromine and sources of "positive bromine" also effected the cyclisation; 231 gave 232 in 65% yield with bromine water while 233 gave 234 in 72% on treatment with N-bromosuccinimide. 3-(4-Hydroxyphenyl)propan-1-ol was transformed to the spiro-oxolan 235 in 25% yield on treatment with 2,4,4,6-tetrabromocyclohexa-2,5-dienone and the same reagent converted the 2-hydroxyaryloxime 236 into the spiro-2,4-dienone 237, which was also produced by cyclodehydration of 238 by boiling pyridine.

1.4.2 Heterocyclic Spirodienones from Quinones and Quinone Methides

Quinones can dimerise to spiro-oxetans as well as syn or anti cyclobutanes, under the influence of light.[142,143] The photo-dimerisation[144] of solid 2,5- and 2,6-dimethyl-1,4-benzoquinones gave the oxetans 239 and 240 in 40 and 70% yields respectively. A study[145] of the molecular packing in quinone crystals has failed to rationalise the type of product formed.

The reaction of quinones with diphenylketene was reported in 1911 by Staudinger and Bereza,[146] who thereby prepared a series of spiro-β-lactones 241 (R^1 = Ph; R^2,R^3 and R^4 = H, Me, Cl or Br) in yields of 30-80%. This work was confirmed in a recent study[147] in which were synthesised analogous compounds 241 (R^1 = Me; R^2 = t-Bu or Me; R^3 = H or Me; R^4 = H) from dimethylketene and the appropriate



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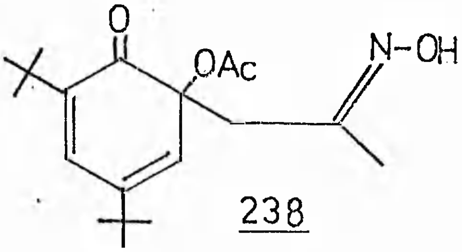

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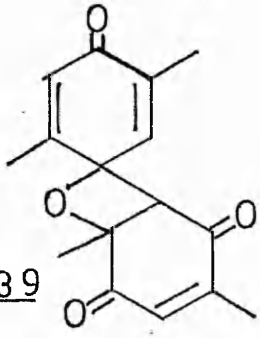

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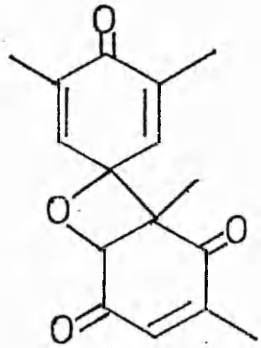

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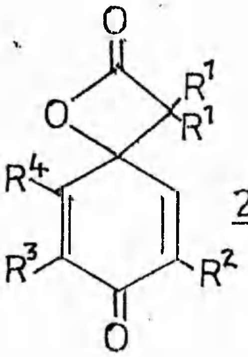

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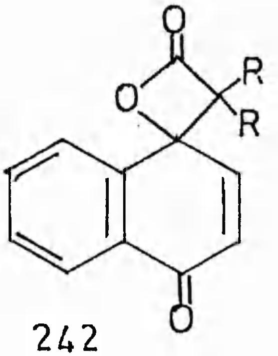

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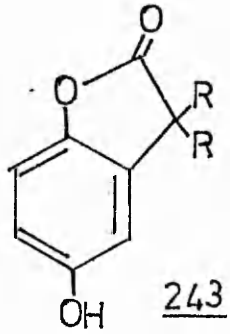

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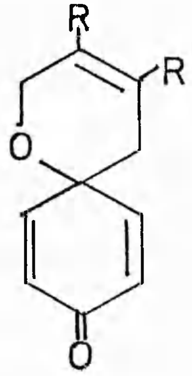

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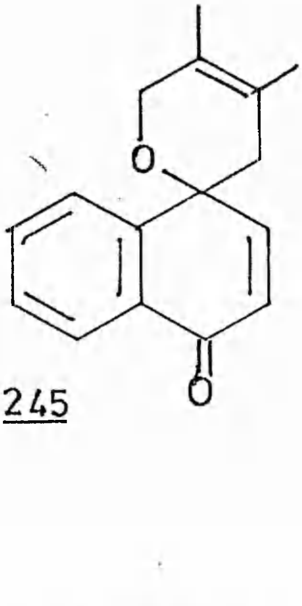

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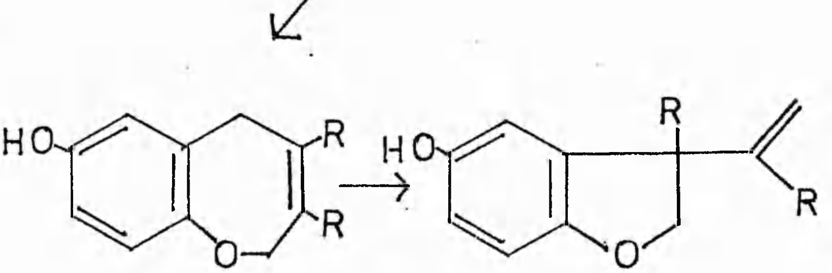

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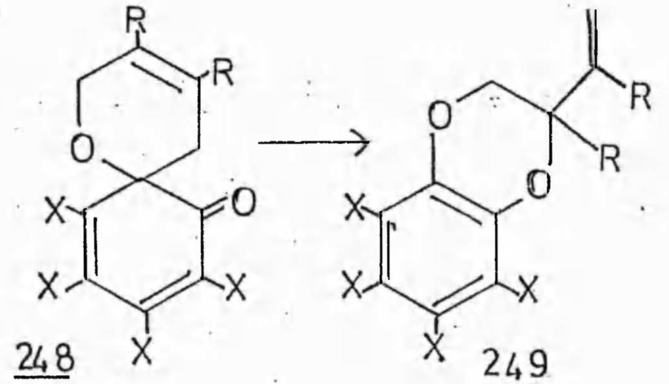
benzoquinone and similarly a spirolactone 242 from 1,4-naphthoquinone. These spirolactones underwent dienone-phenol rearrangements with carbon migration to give benzofuranones 243 in high yield when treated with acetic acid-sulphuric acid.

It was reported[148] that quinones, under the influence of light, add to conjugated olefins to produce spiropyrans; 1,4-benzoquinone reacted with butadiene and with 2,3-dimethylbutadiene to give 244 (R = H or Me) while 1,4-naphthoquinone gave 245 in low yield with dimethylbutadiene. The dienones rearranged on treatment with acetic acid containing a trace of perchloric acid to give the 5-hydroxycoumarans 247 (R = H or Me) by way of the unstable benzoxepins 246. A similar photochemical reaction has been reported[149] between tetrahalo-1,2-benzoquinones and conjugated olefins, to produce spiropyrans 248 (R = Me or Ph, X = Cl or Br) in 10-70% yields. These dienones rearranged to the benzodioxans 249 when heated in benzene.

1,4-Benzoquinone was reported[150] to undergo a photoaddition reaction with unconjugated olefins; reaction with cyclohexene gave 250 and bicycloheptadiene similarly yielded 251. Analogous products were obtained from reactions with cyclooctene, cycloocta-1,5-diene, oct-1-ene, and oct-2-ene in yields of 10-90%. Oct-1-ene produced a mixture of two isomers while oct-2-ene gave four isomers due to the existence of *syn* and *anti* forms as well. Dienone-phenol rearrangements on treatment with ethanolic hydrochloric acid led to carbon migration giving hydroxycoumarans e.g. 252. The product formed from benzoquinone and cyclo-octatetraene was formulated as 253, but a reinvestigation[151] of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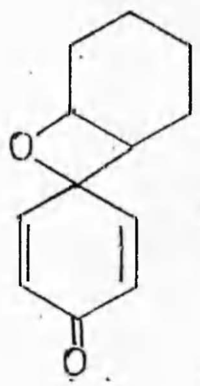
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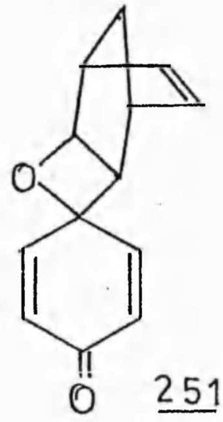
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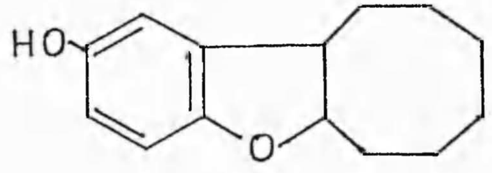
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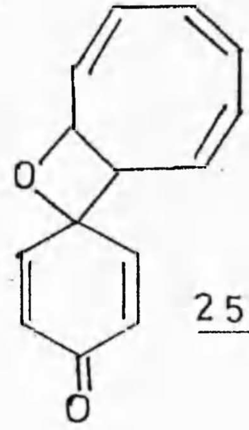
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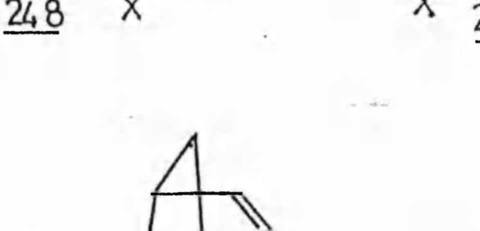
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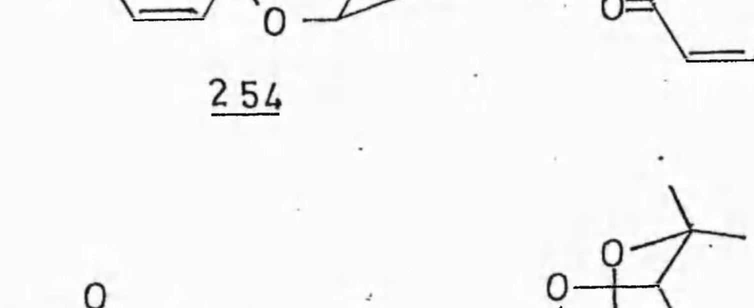
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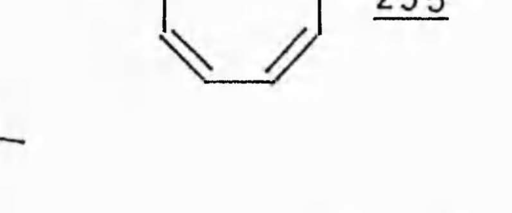
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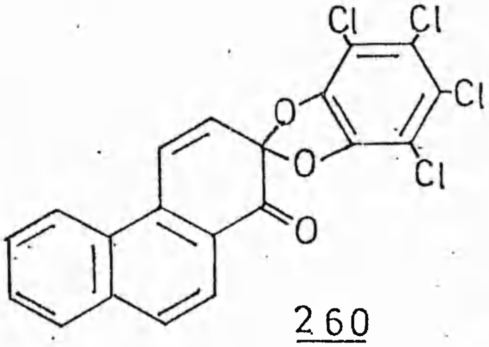
the reaction using a 6W argon laser found that the correct structure was 254 obtained by 1,4-addition. In the presence of oxygen the peroxide 255 was formed in 50% yield; a similar peroxide 257 was produced[152] by irradiation of plastoquinone-1 256 in the presence of oxygen.

Tetrahalo-1,2-benzoquinones react with α-tetralones or naphthols in refluxing benzene to produce spiro-2,4-dienones.[153] Thus 258 (R = H or OMe) is formed from the reaction with the appropriate α-tetralone or naphth-1-ol. Naphth-2-ol under the same conditions produced a mixture of 258 (R = H) and a small amount of 259. Similarly 3,4-dihydrophenanthren-1-(2H)-one formed 260 and 3-methoxyphenol gave 261. The authors propose that when a ketone is the starting material it is first dehydrogenated to the phenol. In a similar reaction[154] the azoketone 262 reacts with o-chloranil or o-bromanil to give the spirodienone 263 (X = Cl or Br) in 31 and 51% yields respectively.

Addition reactions of the quinone carbonyl group can also lead to dienones. Treatment[155] of 2,6-dimethoxy-1,4-benzoquinone with diazoethane yielded 264 and 1,4-benzoquinone reacted[156] with the phosphorane 265 (a carbon nucleophile) to form 266. Phenylisocyanate is reported[157] to add to 1,2-benzoquinone to give 267.

Two syntheses of one of the simplest heterocyclic spirodienones, 1,4-benzoquinone monoethylene ketal 268 have recently appeared. The first[158] proceeded from the bis(ethylene ketal) of cyclohexa-1,4-dione which was brominated and dehydrobrominated to give 1,4-benzoquinone



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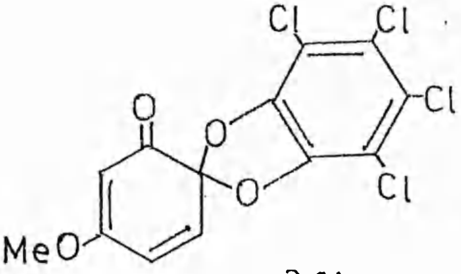

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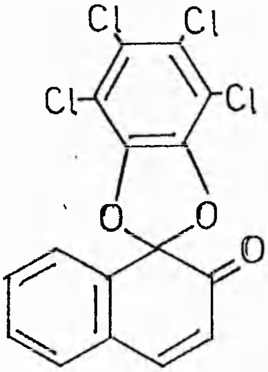

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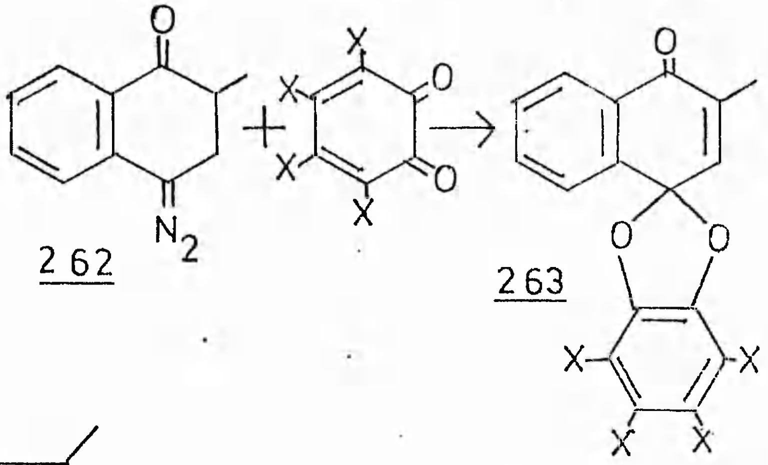

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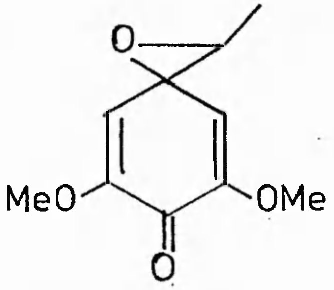

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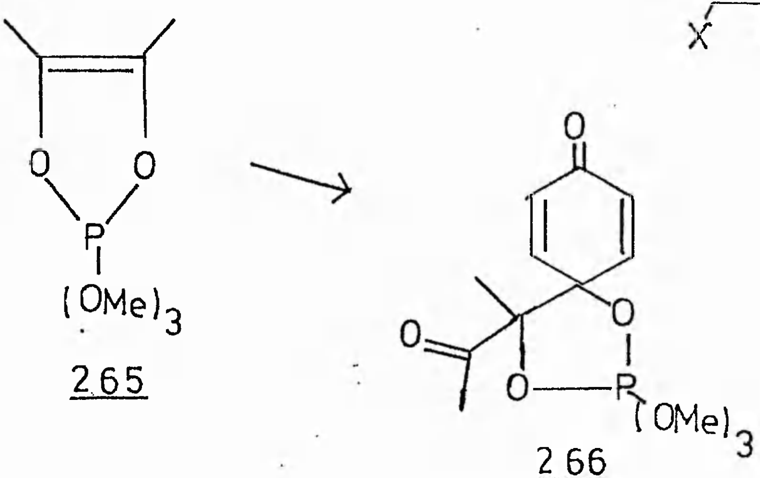

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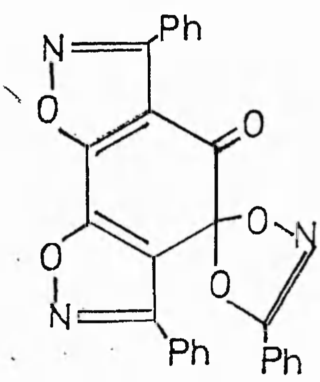

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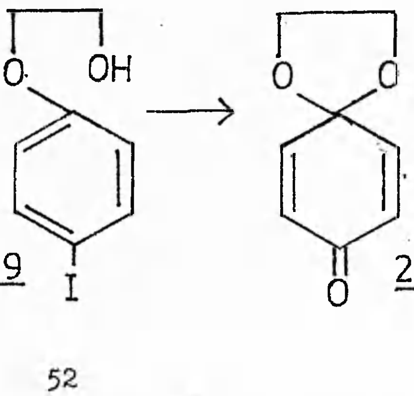

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bis(ethylene ketal) which on partial hydrolysis gave 93% of 268. The second synthesis[159] is an example of an Ar_1-5 participation reaction (see Section 1.3.1). 2-Phenoxyethanol was iodinated to give 269 which on irradiation afforded 268 in 84% yield, provided the reaction was carried out on a small scale.[160]

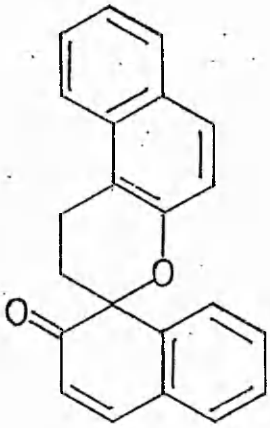
Spirodienones are produced by the dimerisation of quinone methides. Chauhan et al[161] prepared 1,2-naphthoquinone-1-methide by heating 1-dimethylaminomethyl-2-naphthol and found that it existed as the dimer 270, dissociating into the monomer at 160-200°. However a later report[162] found that naphthoquinone methide dimer changed to an isomeric spirodienone 271 on heating above 80°.

1.5 Spirodienones in Natural Products

The spirocyclohexadienone structure occurs in two main classes of natural products, fungal metabolites and alkaloids. It also occurs in an oxidation product of vitamin E, which is thought to be a liver metabolite.

1.5.1 Vitamin E Oxidation Product

Vitamin E or α-tocopherol 272 ($R = C_{16}H_{33}$) was first oxidised to the dimeric product with alkaline ferricyanide by Martius and Eilingsfeld[163] in 1959, and the structure of the product was investigated by many workers before being determined[164] as 273, one of the four possible spirodienone isomers. Another ferricyanide oxidation[165] of α-tocopherol yielded 32% of 273 together with a small amount of a trimeric product formulated as 274. Similar studies were carried out[165] on the model compound 272



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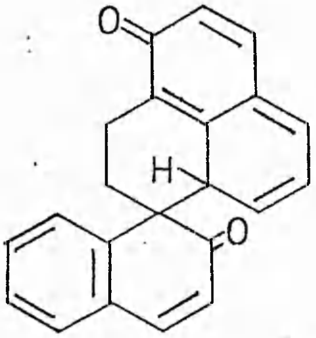

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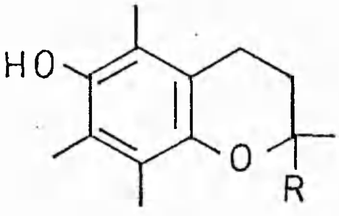

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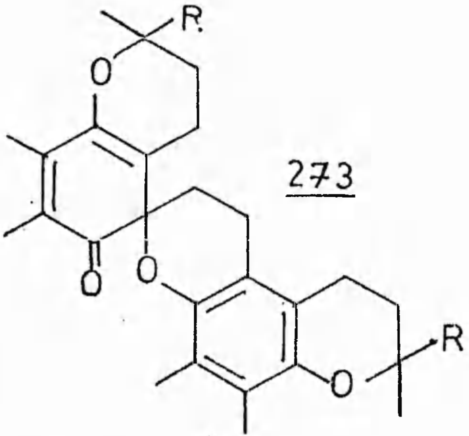

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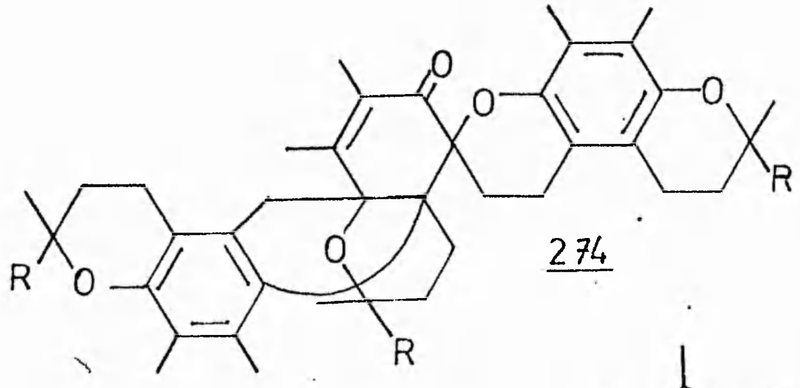

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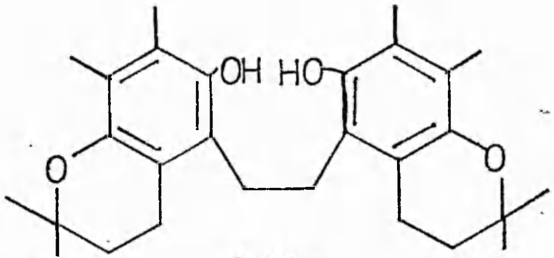

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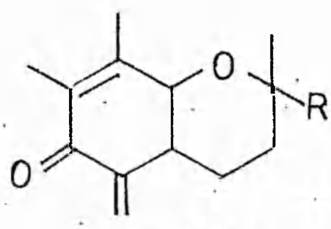
(R = Me) and it was proposed that the initial step in the oxidation was the formation of the diphenylethane 275, which then underwent intramolecular coupling. Later it was reported[166] that a more likely mechanism is the initial formation of a 1,2-quinone methide 276 which then dimerised.

It was noticed[167] that the n.m.r. spectrum of the dimer showed loss of identity between the vinylic and aromatic methyl groups on raising the temperature and this was explained[168] by acid-catalysed ring opening to a phenoxonium ion and subsequent recyclisation (Scheme III). Dilute trichloroacetic acid was sufficient to achieve this and the result was used to explain the otherwise somewhat puzzling reduction of the dimer to the diphenylethane 275 by ascorbic acid.[169] An oxidative dimerisation to a compound 277, similar to the vitamin E oxidation product, has recently been reported.[170]

1.5.2 Fungal Metabolites

Griseofulvin (from Penicillium species)

Griseofulvin is one of the few medically important anti-fungal agents. Its gross structure was determined[171] as 278 and its absolute configuration subsequently established.[172] It is a reduced spirodienone but is easily oxidised to dehydrogriseofulvin 279 by selenium dioxide.[173] Compound 279 has been synthesised from griseophenone A 280 by several workers, in 60% yield by alkaline ferricyanide oxidation[174] and quantitatively by either lead dioxide or manganese dioxide in ether-acetone.[175] Analogues of griseofulvin have been prepared[174,176] *via* the oxidation of the appropriate benzophenones to the dehydrogriseofulvins 281



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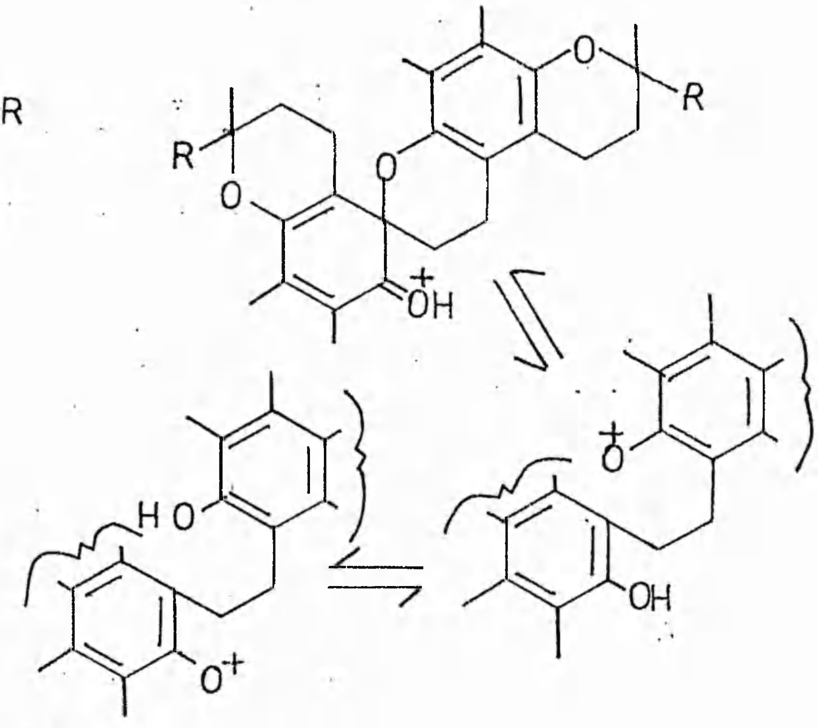
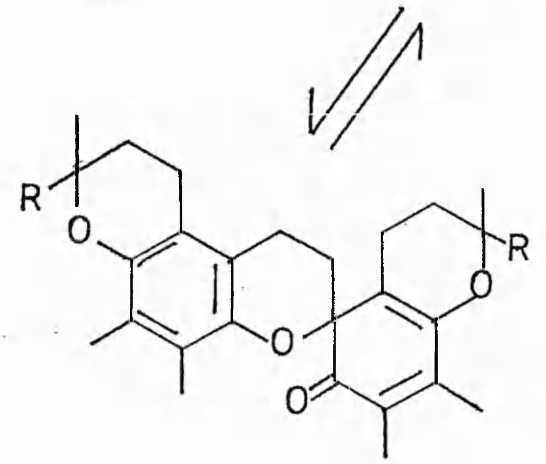
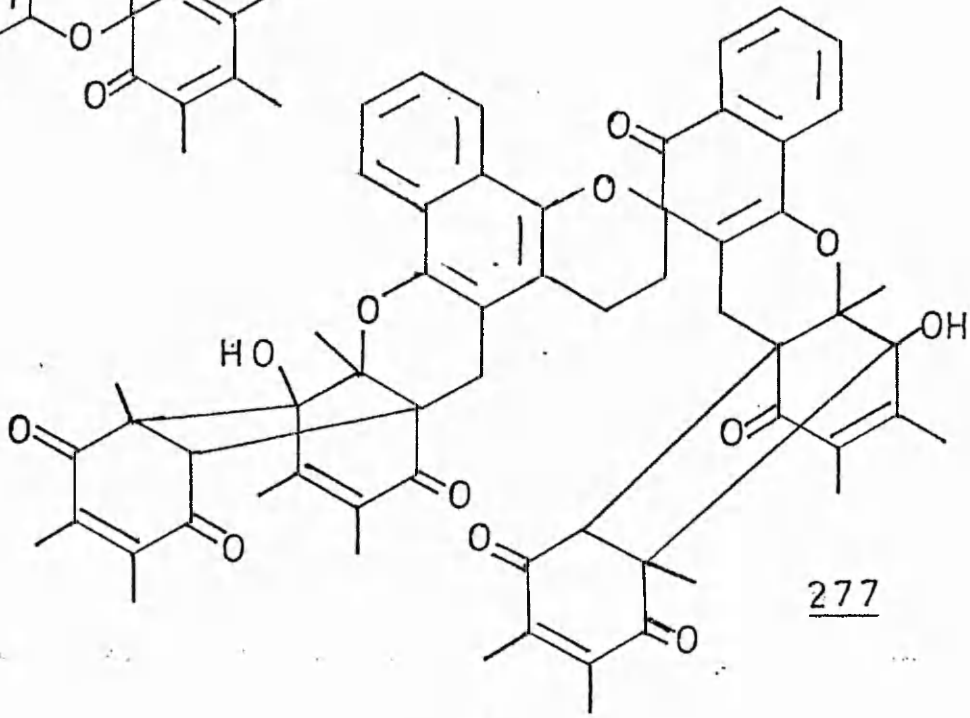


Scheme III





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(R^1 = H, F, Cl or Br; R^2 = H or Cl; R^3 = H or Me; R^4 = OMe). Curtis et al[120] have postulated that bulky substituents in the 6 and 2′ and/or 6′ positions favour oxidative coupling of the benzophenones.

The carboxylic acid 282 obtained by acid hydrolysis of 279 has been oxidised to dehydrogriseofulvoxin 283 in 85% yield by manganese dioxide in acetone-ether.[177] It has not been ascertained whether the griseofulvoxin structure occurs naturally.

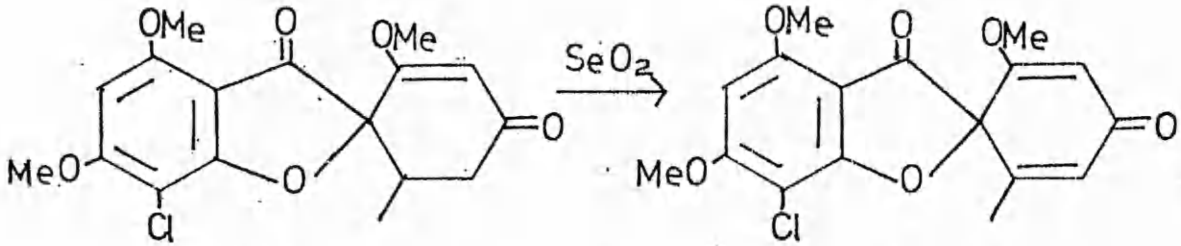
Geodin and Erdin (from Aspergillus terreus)

These structures were determined as 284 (R = H) and 284 (R = Me) respectively by Barton and Scott.[178] Geodin methyl ether was prepared[173] in 33% yield by alkaline ferricyanide oxidation of 6-O-methyldihydrogeodin 285. On treatment with 80% sulphuric acid geodin and erdin furnish[179] their respective hydrates 286 (R = H or Me) which are precursors of a second class of compound.

Geodoxin (from Aspergillus terreus)

Geodoxin has the structure[180] 287. It has been synthesised[181] in 88% yield from geodin hydrate 286 (R = H) by oxidation with lead dioxide in ether.

Similarly from Oospora sulphurea-ochracea the compounds sulochrin 288, dechlorogeodin 289, asterric acid 290 and dechlorogeodoxin 291 have been isolated.[182] These compounds have been interconverted in vitro, with 288 giving 289 in 20% yield on treatment with alkaline ferricyanide[183], and 291 formed from 290 in 71% yield by oxidation with lead dioxide in ether.[181]



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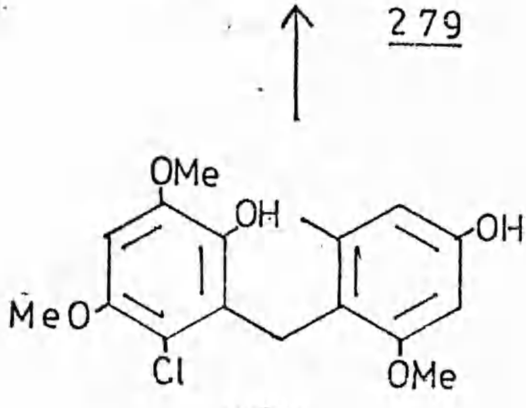

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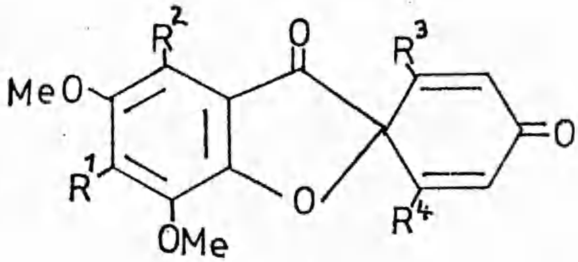

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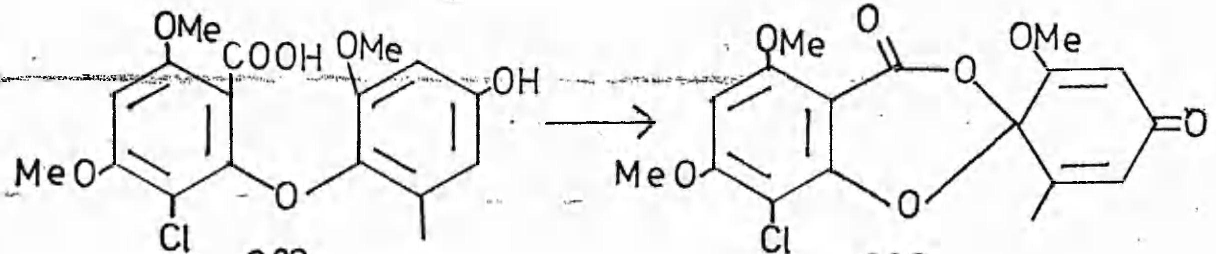

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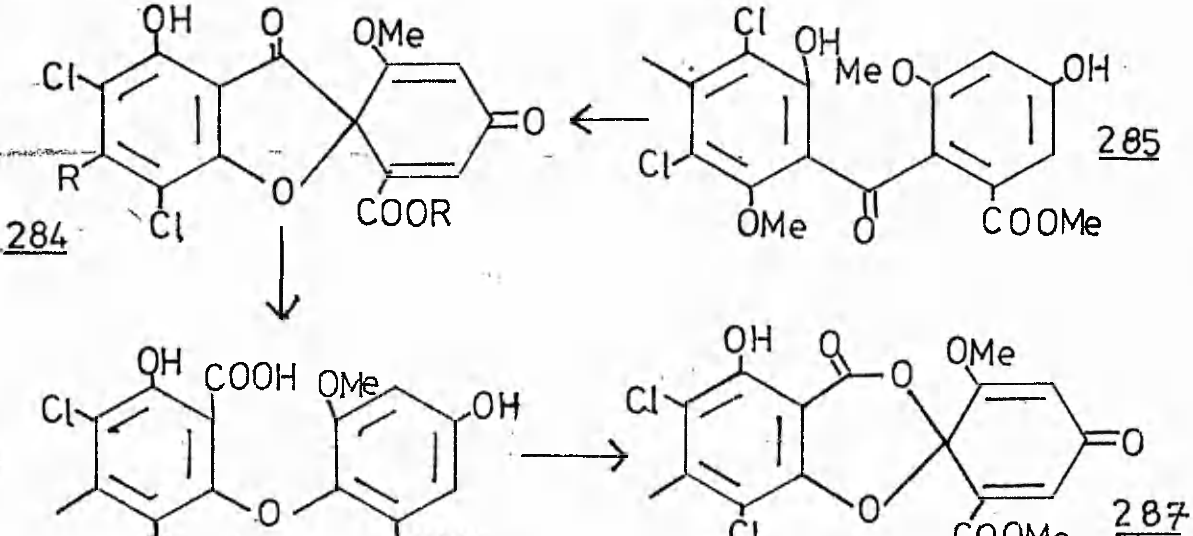

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Picrolichenic acid (from Pertusaria amara)

Its structure was determined[184] as 292, and confirmed by a synthesis[185] in 25% yield, in which the phenolic ester 293 was treated with 15 equivalents of manganese dioxide in benzene. No trace of o-p coupling to give the isomeric compound 294 was observed.

Thelepin (from Thelepus setosus)

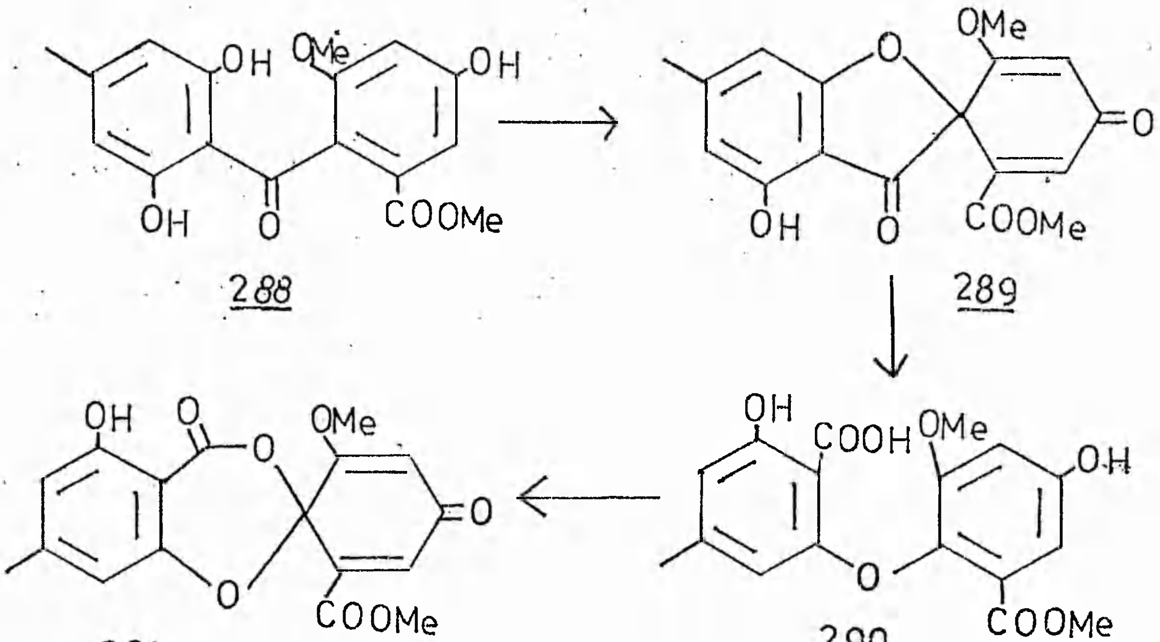
This compound, similar to the fungal metabolites, was recently isolated from a marine annelid, and its structure established[186] as 295. The diphenylmethane, thelephenol 296 also occurs in the organism but attempts to convert it to thelepin by oxidation with alkaline ferricyanide or lead dioxide were unsuccessful. Thelepin shows antifungal activity comparable with that of griseofulvin.

1.5.3 Alkaloids

The number of instances in which the spirocyclohexadienone structure is found either in naturally occurring alkaloids, or in synthetic intermediates, both in vivo and in vitro is substantial and this review will be illustrative rather than comprehensive.

Proaporphine alkaloids

These are a group of alkaloids[187] (about 30 in number) derived from 1-benzyltetrahydroisoquinolines by intramolecular oxidative coupling and typified[188] by pronuciferine 297. Proaporphines have been synthesised by classical methods,[189,190] by the Pschorr cyclisation,[191] by photo-dehydrohalogenation[192] and by phenol oxidative coupling. Battersby et al[193] were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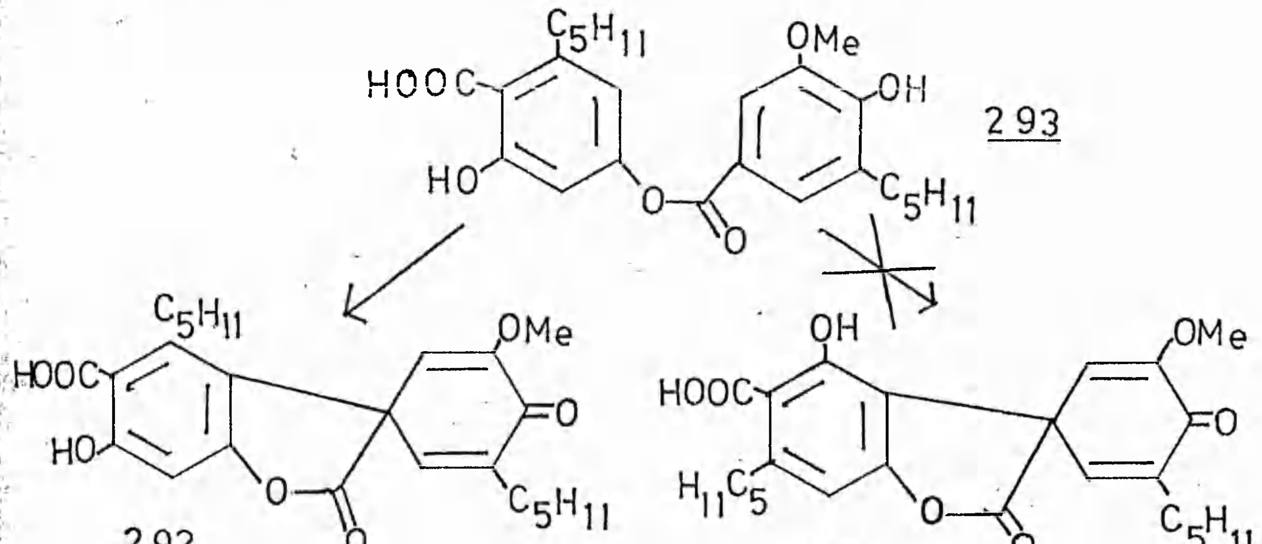
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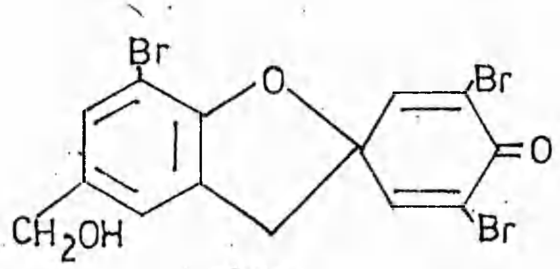
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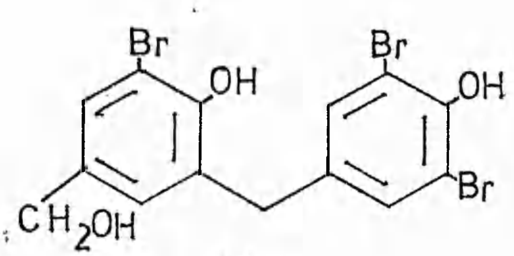
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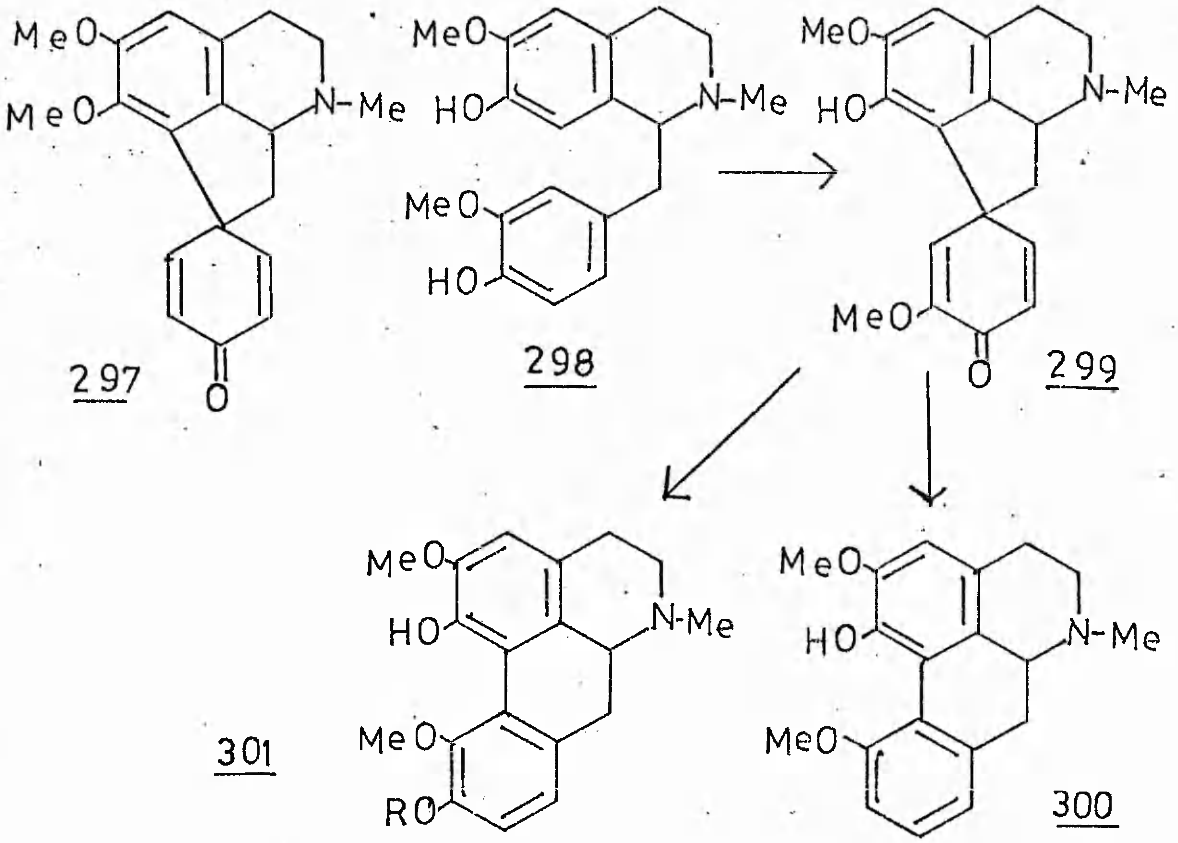
first to duplicate the proposed[1] biosynthetic pathway when they converted orientaline 298, by two phase ferricyanide oxidation to the proaporphine, orientalinone 299 (found as a natural product two years after this work). Reduction, by borohydride, to the dienol followed by dienol-benzene rearrangement in methanolic hydrochloric acid gave[193] the aporphine, isothebaine 300, while dienone-phenol rearrangement[194] of 299 with a mixture of acetic and hydrochloric acids yielded isocorytuberine 301 (R = H) and with methanolic hydrochloric acid produced corydine 301 (R = Me).

Spiro-2,4-dienones related to proaporphines have been prepared; e.g. two phase ferricyanide oxidation[194] of 7-hydroxy-1-(2′-hydroxy-4′,5′-dimethoxybenzyl)-6-methoxy-2-methyl-1,2,3,4-tetrahydroisoquinoline gave a diastereoisomeric mixture of dienones 302.

Ferricyanide oxidation[195] of the protosinomenine derivative 303 produced another spirodienone system 304 which is an intermediate on a biosynthetic pathway[196] to aporphines. Compound 304 is also called proerythrinadienone due to its importance in the biosynthesis of Erythrina alkaloids (see p. 65). A recent report[197] details a photochemical synthesis of 304 and its photo-rearrangement to the aporphine, boldine.

Homoproaporphines are similar to proaporphines but derived from 1-phenethylisoquinolines. The only naturally occurring member is kreysiginone[198] 305, although several analogues have been prepared[199,200] by oxidation of the corresponding



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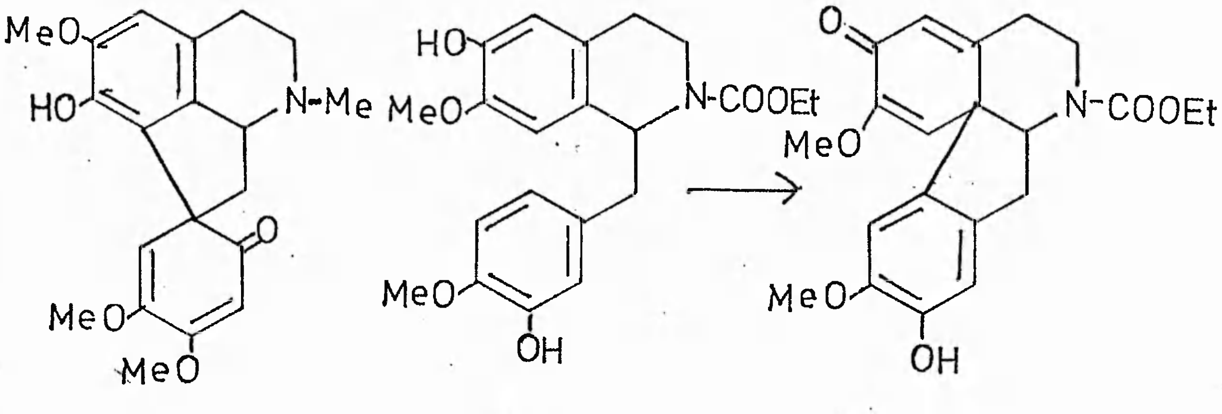

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phenethylisoquinolines; a recent report[201] describes the use of anodic oxidation to produce homoproaporphines in yields of up to 40%.

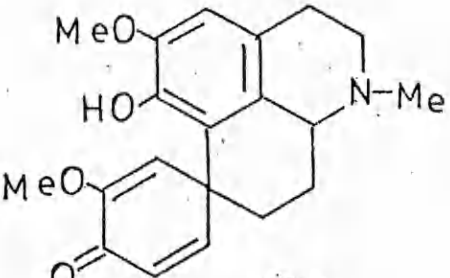
Homoproaporphines undergo dienone-phenol and, after reduction, dienol-benzene rearrangements to homoaporphines in the same way as proaporphines.[202] Oxidation of a 1-phenylpropyltetrahydroisoquinoline derivative with ferricyanide produced[203] a spirodienone 306.

Morphinandienone alkaloids

A morphinandienone 307 (R^1 = OH, R^2 = H) was synthesised[204] in 1963 from thebaine and was later found to be a natural product, salutaridine, a biosynthetic precursor of morphine. Considerable work was carried out on the oxidative coupling of reticuline 308 to give salutaridine (para-ortho coupling), rather than pallidine[205] 307 (R^1 = H, R^2 = OH, para-para coupling) or the aporphine, isoboldine (ortho-para coupling).

The desired reaction was first achieved[206] using a two phase ferricyanide oxidation to give salutaridine in 0.03% yield (determined by a radiodilution technique). A later oxidation[207] using thallium trifluoroacetate improved the yield to 23%. Morphinandienones have recently been prepared[201,208-10] in high yields by anodic oxidation of both phenolic and non-phenolic 1-benzyltetrahydroisoquinolines. A second class of morphinandienones are diastereoisomers of salutaridine and its derivatives, and are typified by sinoacutine[211] 309.

A synthesis of the unusual dibenzazonine alkaloid



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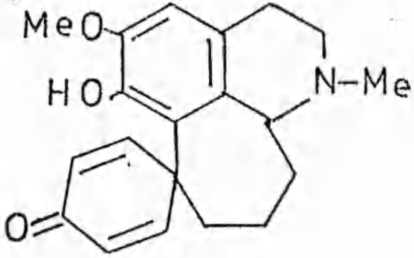

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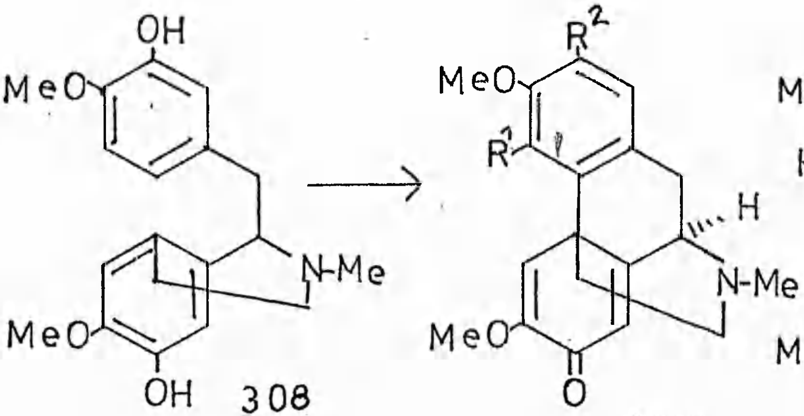

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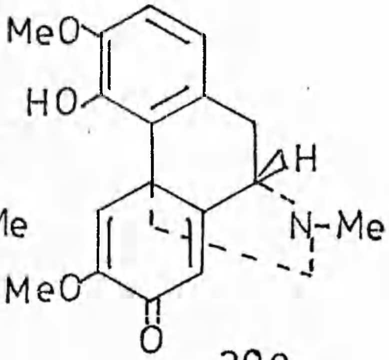

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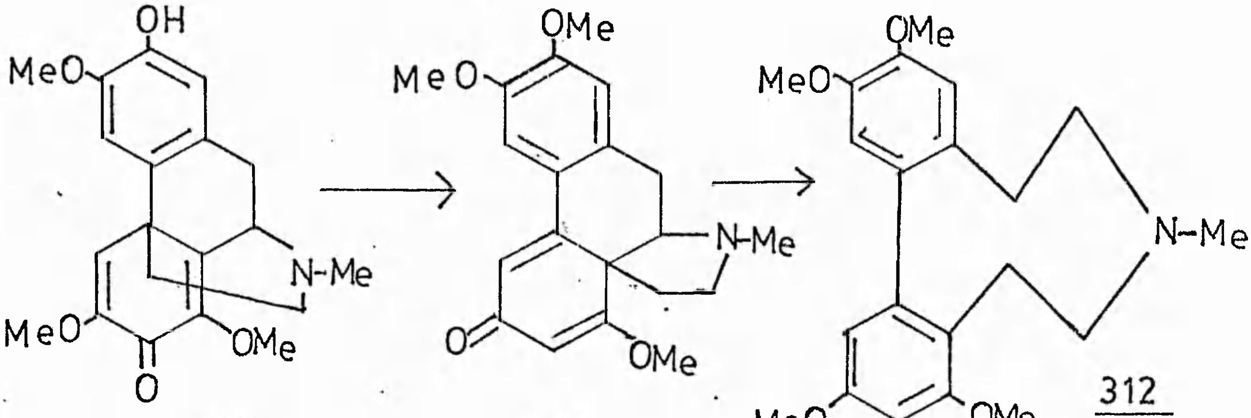

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312

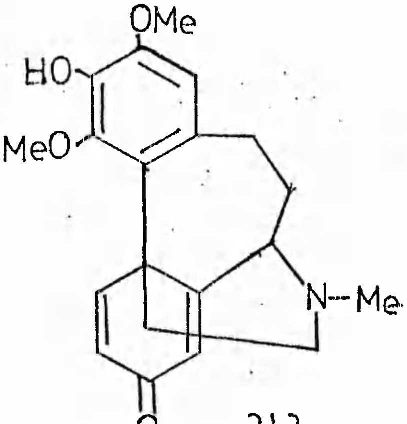
protostephanine 312 (having two methoxy group *meta* to one another) was reported,[212] in which a 1-benzylisoquinoline was oxidised to the morphinandienone 310; this after etherification, reduction and dienol-benzene rearrangement gave the spirodienone 311 which could be converted to 312.

Homomorphinandienones are known, and an example is androcymbine[213] 313. A derivative of androcymbine has been obtained[214] by a two phase ferricyanide oxidation of a 1-phenethyltetrahydroisoquinoline, homoreticuline. Androcymbine has been found[215] to be a biosynthetic precursor of colchicine.

Erythrina alkaloids

Although none of the naturally occurring members of this class is a spirodienone, their syntheses and biosyntheses[216] proceed through an important intermediate, erysodienone 314. Erysodienone was prepared[217] in 35%yield by oxidative coupling of the bis-(phenethyl)-amine 315 and this led to 315 being proposed[217] as a biosynthetic precursor. Later work,[218] however, showed that the 1-benzyltetrahydroisoquinoline, N-norprotosinomenine 316 was a precursor and the mechanism of its conversion was suggested to be as shown (Scheme IV).

An erysodienone type compound 320 has been prepared[219] from a 1-benzylisoquinoline, although not by the biogenetic route. Compound 317 on treatment with vanadium oxytrichloride was converted to the morphinandienone 318 which then led to the dibenzazonine 319, oxidation of which, by ferricyanide, gave 320. Recently, the first two steps of Scheme IV have been carried



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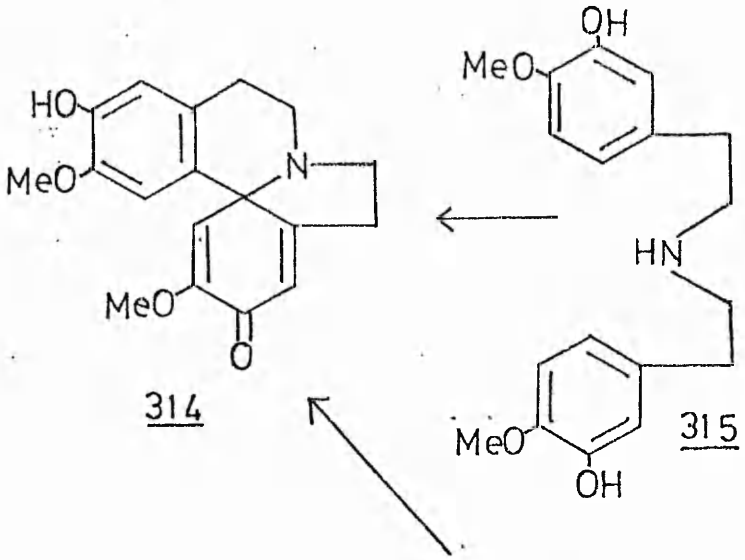

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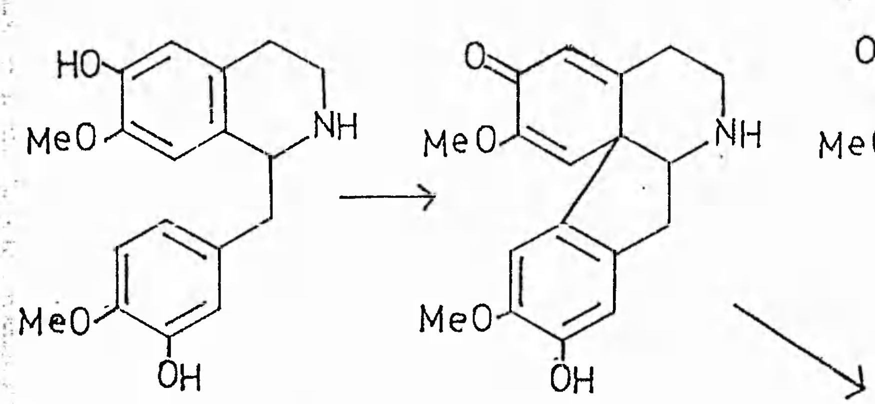

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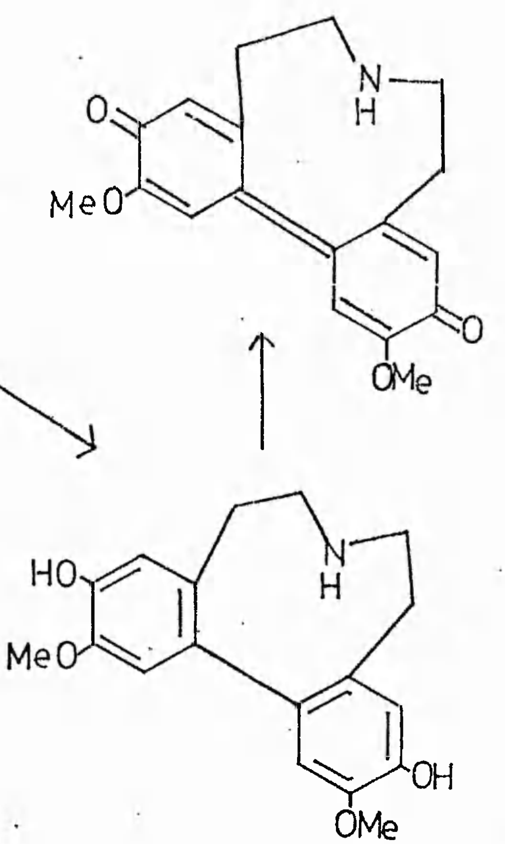
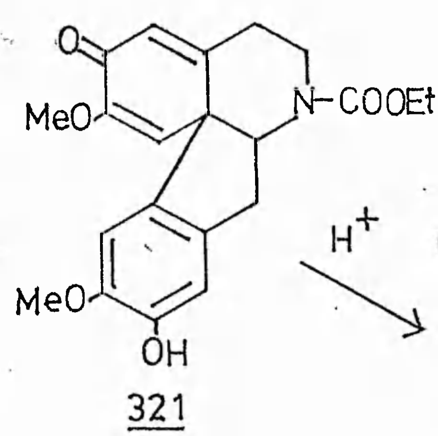
Scheme IV





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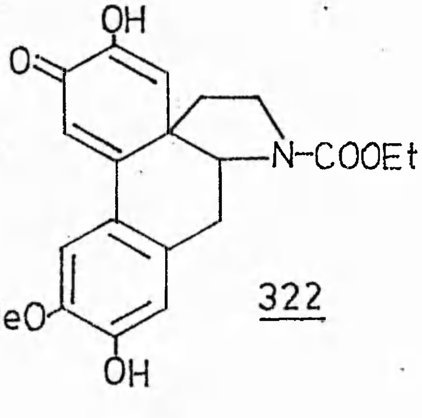
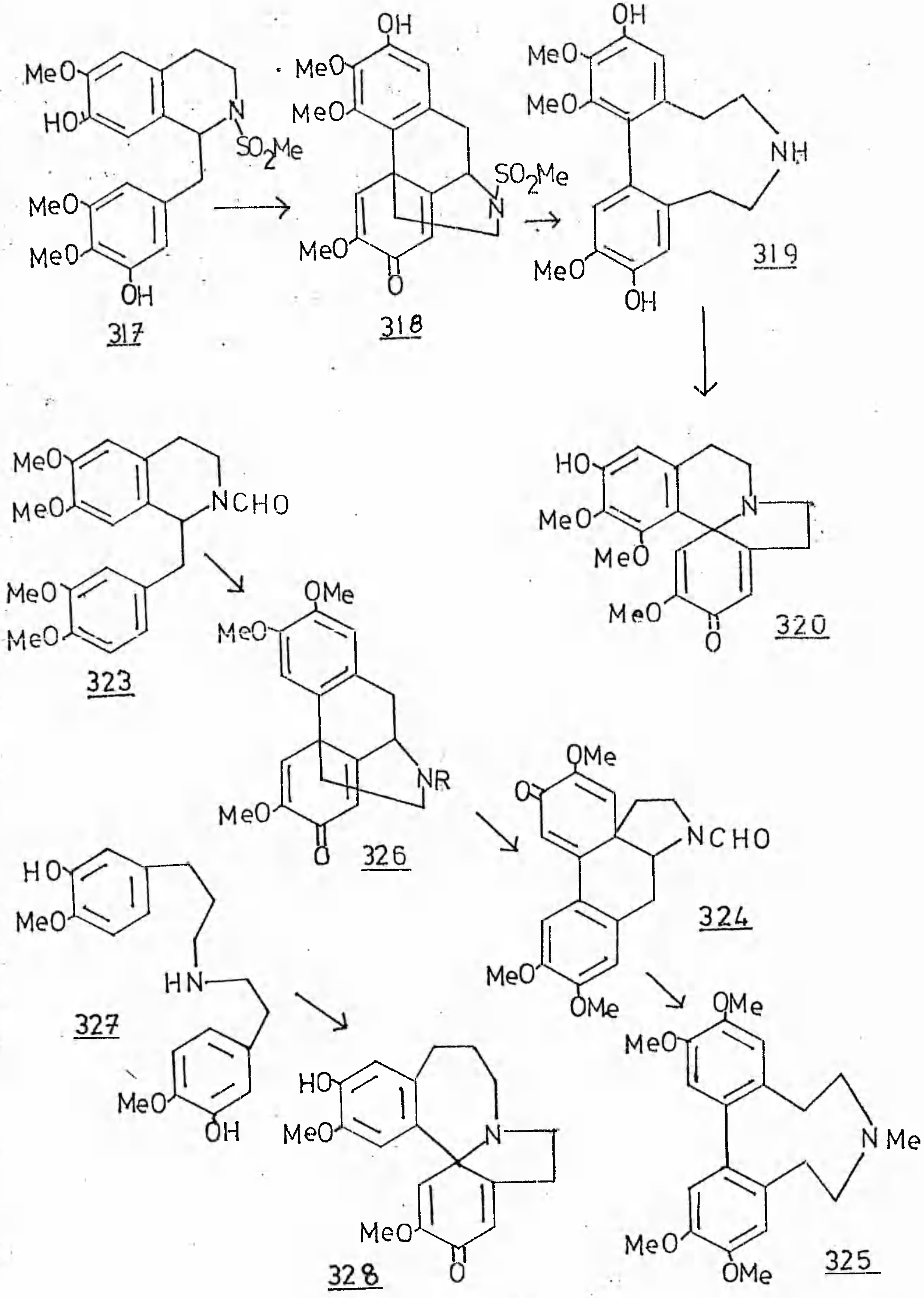


322

out *in vitro*.[220] N-trifluoroacetyl-N-norprotosinomenine on oxidation with vanadium oxytrichloride gave the N-trifluoracetyl-proerythrinadienone which was then converted to the dibenzazonine.

A novel spirodienone structure 322 was formed[221] by acid-catalysed rearrangement of the proerythrinadienone 321, obtained by oxidation of the protosinomenine-type precursor. Recent work[222] has shown that treatment of N-formyllaudanosine 323 with vanadium oxytrifluoride in trifluoroacetic acid leads to a small amount of the aporphine, N-formylglaucine and 55% of a dienone 324 of similar structure to 322. Compound 324 was easily converted to the dibenzazonine 325 by acid-catalysed rearrangement followed by methylation. It was later found[223] that the oxidation of N-formyllaudanosine proceeded through the morphinandienone 326 (R = CHO) and that O-methylflavinantine 326 (R = Me) was also converted[224] to the dibenzazonine 325 by acid-catalysed rearrangement and subsequent reduction.

The biogenesis of homoerythrina alkaloids is proposed to parallel that found for erythrina alkaloids.[225] Homoerythrinadienone 328 has been prepared[226] by ferricyanide oxidation of the amine 327; a later report[227] details a biogenetic synthesis in which the homoproerythrinadienone 329 on treatment with base was converted into the dibenzazecine 330 which, on ferricyanide oxidation, gave a mixture of 328 and the isomeric homoerythrinadienone 331 in 15 and 45% yields. Another synthesis[228] featured the novel oxidative coupling of the phenylacetylquinoline 332, by ferricyanide, to give a 67% yield of the dibenzoquinolizine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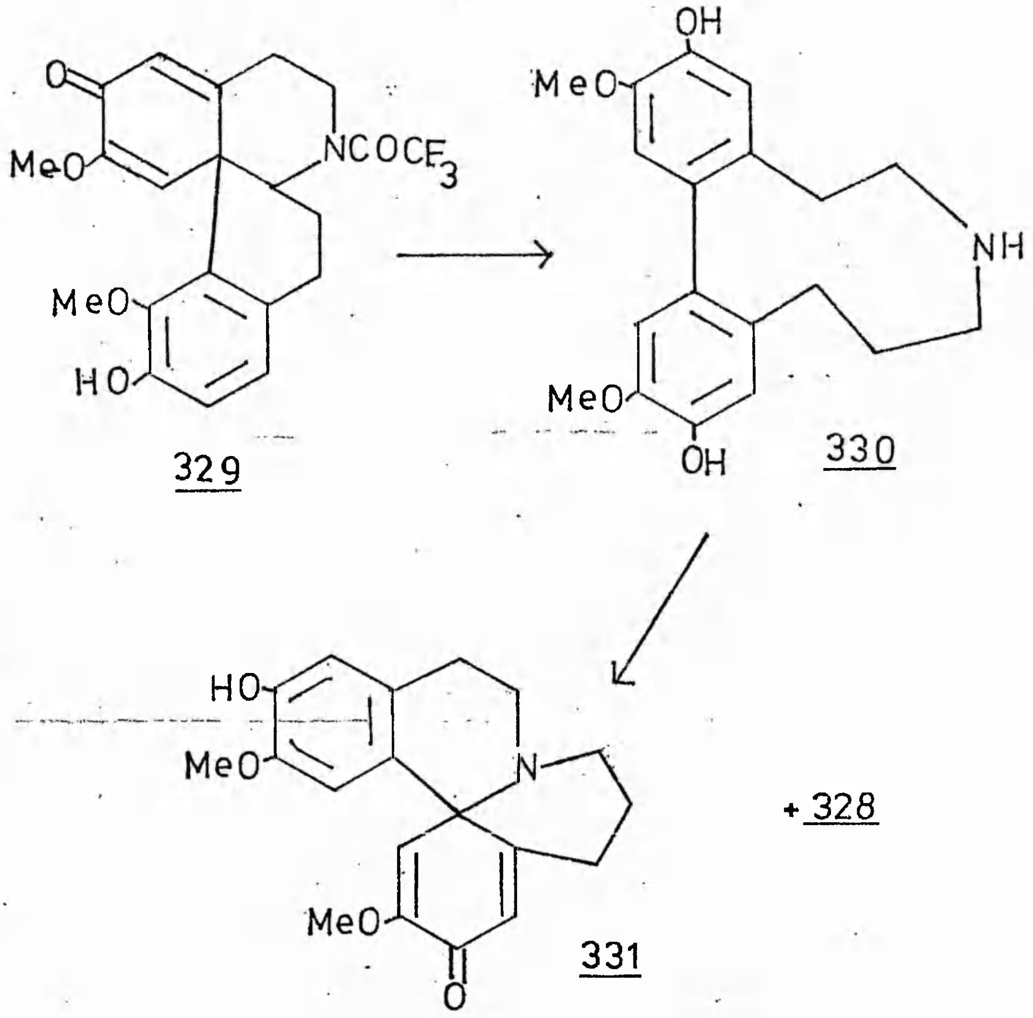
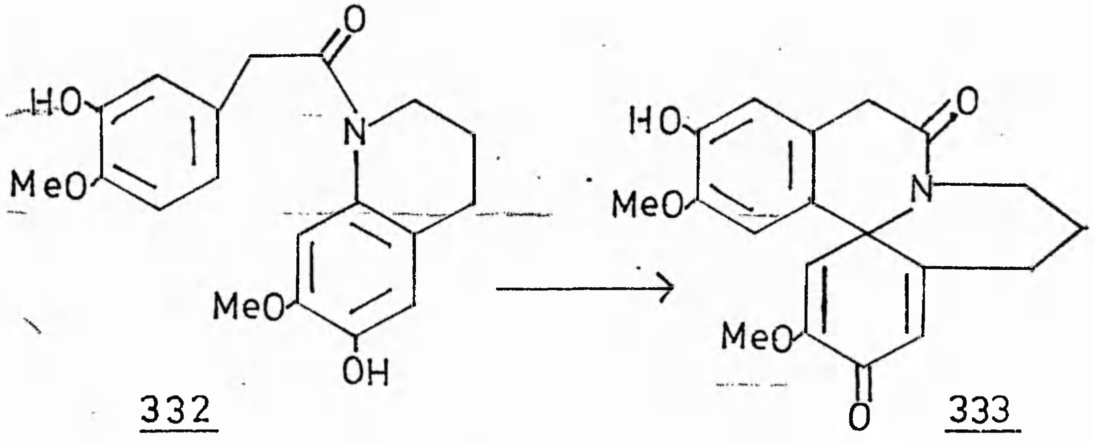

MeO
NCOCF3
MeO
HO
329
OH
MeO
NH
MeO
OH
330
HO
MeO
N
MeO
O
331
+ 328


O
HO
N
MeO
MeO
OH
332
HO
O
N
MeO
MeO
O
333

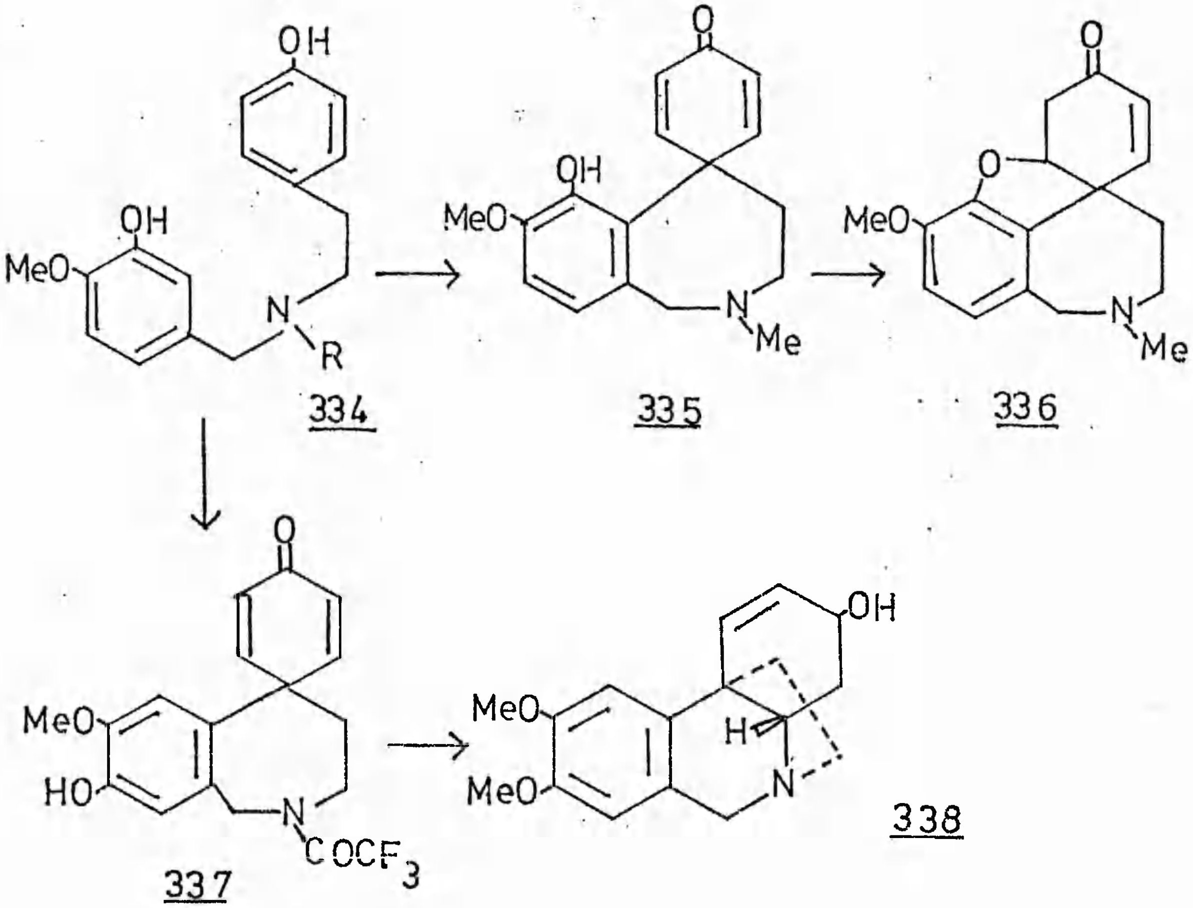
dienone 333 which was then reduced to 328.

Amaryllidaceae alkaloids

Three classes of Amaryllidaceae alkaloids are known, two of which are biosynthetically derived from spirodienones. The common precursor[229] for all three classes is the norbelladine-type compound 334 (R = H or Me). In 1962 the oxidation of 334 (R = Me) with ferricyanide or manganese dioxide was reported[230] to yield the *ortho-para* coupled product 335 which tautomerised to the alkaloid, narwedine 336. Some time later, the *para-para* coupling of 334 ($R = COCF_3$) was achieved[231] using vanadium oxytrichloride to give the spirodienone 337 which was then converted to the alkaloid maritidine 338. Anodic oxidation of 339 afforded[232] the spirodienone 340 from which the alkaloid oxocrinine was easily obtained. Recently,[233] anodic oxidation of the 3-benzyloxindole 341 produced the spirodienone 342 in 15% yield.

Cularine alkaloids

The four members of this group are typified by cularine itself 343, and there has been speculation that the biogenesis of cularine proceeds by dienone-phenol rearrangement of a "procularine" spirocyclohexadienone 344 (R = H). Kametani *et al*[234] prepared the dienone 344 (R = OMe) in low yield by ferricyanide oxidation of the 1-benzyltetrahydroisoquinoline 345 (R = OMe) and found[235] that, on treatment with concentrated sulphuric acid, it rearranged by oxygen migration to the cularine-type compound 346. The dienone 344 (R = H) was synthesised,[236] as a mixture of diastereoisomers, by ferricyanide oxidation of the isoquinoline



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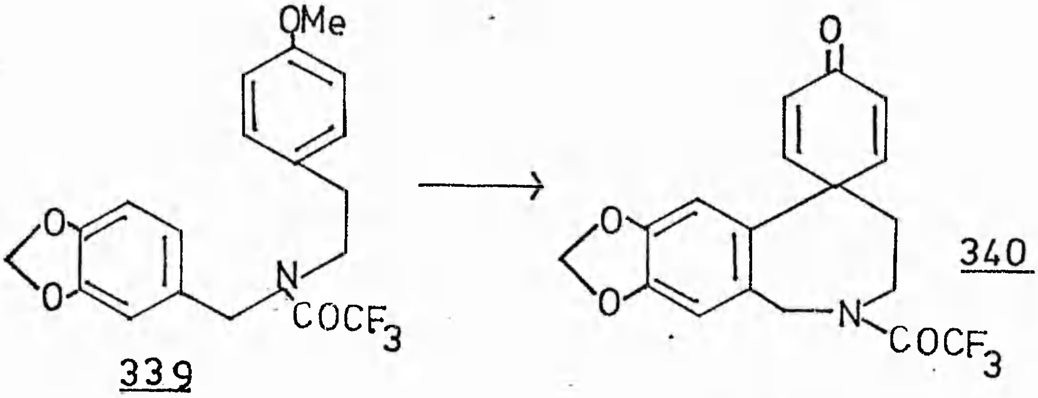

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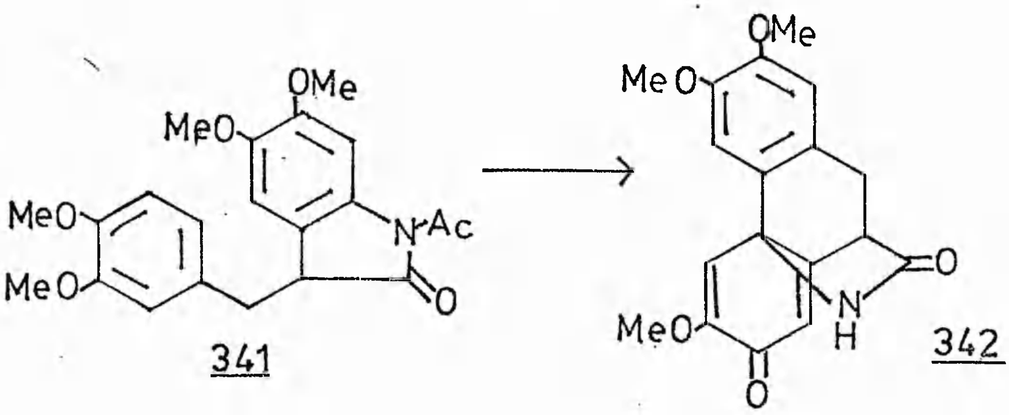
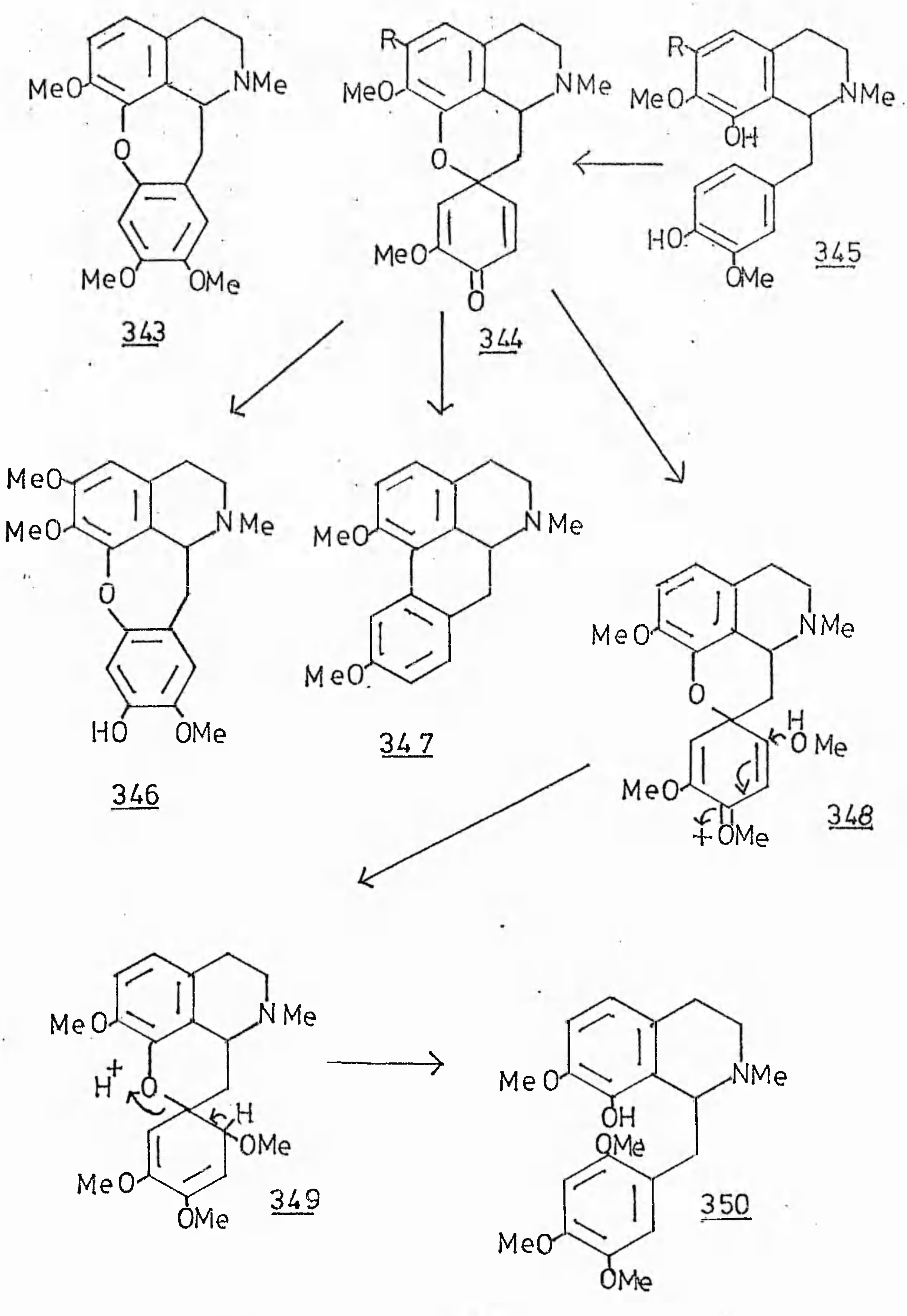


341 342

345 (R = H) and one group[237] found that it rearranged to the aporphine 347 with concentrated sulphuric acid, while another[238] used hydrogen chloride in methanol and isolated the 1-benzyltetrahydroisoquinoline 350; this was found to have arisen *via* intermediates 348 and 349.

Phenanthroquinolizidine alkaloids

The alkaloid cryptopleurine 354, a member of this group, has been synthesised[239] via a spirodienone. The quinolizidinone 351 (R = H) was oxidised with manganese dioxide to the dienone 352 (R = H), which, on dienone-phenol rearrangement with acetic anhydride-sulphuric acid yielded 353; subsequent hydrolysis, methylation and reduction gave cryptopleurine 354. The oxidation step has also been carried out anodically[240] on the fully methylated compound 351 (R = Me) to give the dienone 352 (R = Me) in 60% yield.



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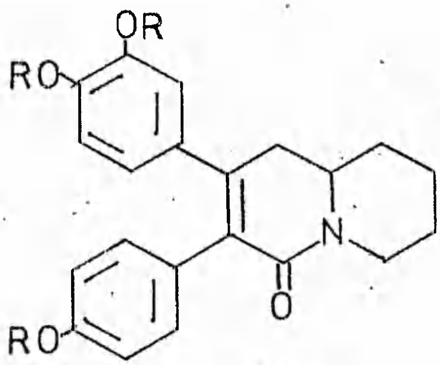
CHAPTER TWO

The Synthesis of Diarylamines and N-Acyldiarylamines

2.1 Introduction

In view of the successful oxidation of 2,4′-dihydroxydiphenylalkanes[112,113] and 2,4′-dihydroxydiphenyl ethers[115,116] to spirodienones, it was thought likely that oxidation of 2,4′-dihydroxydiphenylamine derivatives 355 (R = H or acyl) would, by intramolecular coupling, furnish a novel nitrogen-containing spirocyclohexa-2,5-dienone 356. An N-acyl derivative was particularly desired in order that the lone pair of electrons on the nitrogen atom should be protected during the oxidation to avoid undesirable side reactions. Therefore it was necessary to synthesise diarylamines and their N-acyl derivatives substituted in the 2- and 4′-positions with groups which could subsequently be converted to hydroxyl functions.

Diarylamines are a class of compounds that have no entirely satisfactory, general preparative method.[241,242] Aryl halides are relatively insensitive to nucleophilic substitution[243] and will usually react only when one or more electron-attracting groups are substituted ortho or para to the halogen atom. Nitro groups are particularly effective as activating substituents and 2,4-dinitrohalobenzenes will react with arylamines fairly readily. Unactivated aryl halides can sometimes be induced to react with arylamines by catalysis with copper or copper salts;[244,245] this is known as the Ullmann condensation. A related reaction, also catalysed by copper compounds, is that between aryl halides and



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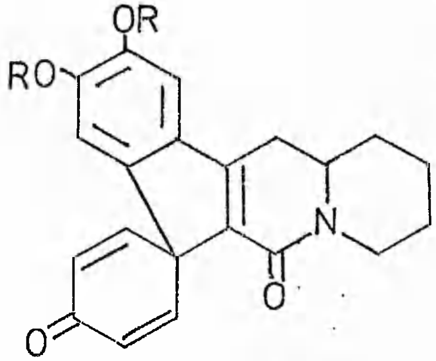

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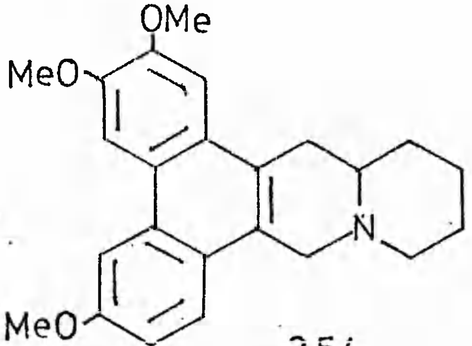

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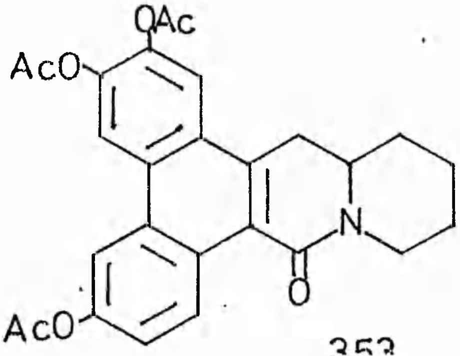

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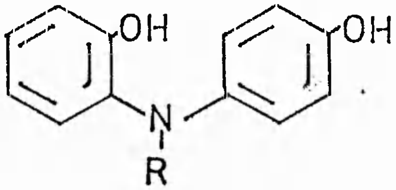

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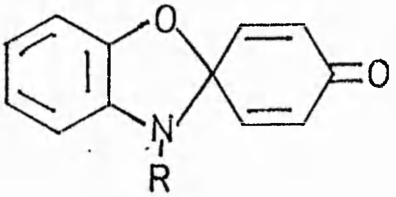

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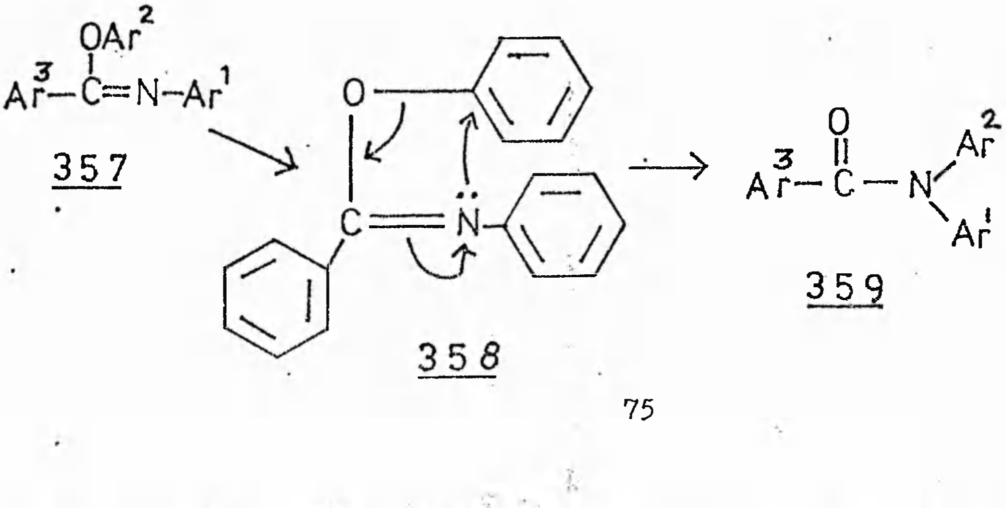


N-arylacetamides, known as the Goldberg reaction. These reactions have received attention[246,247a] largely as routes to diphenylamine-2-carboxylic acids which can then be converted to 9-acridones and have been reviewed elsewhere.[243,248]

Various factors are known to influence the reaction.

1. The aryl halide should bear an electron-withdrawing group in the <u>ortho</u> or <u>para</u> position; 2-carboxyaryl halides are often used, with the initially formed diarylamine-2-carboxylic acids undergoing subsequent thermal decarboxylation if the free diarylamine is desired.[249,250]

2. Catalysis can be offered by various forms of copper or its salts, but "Naturkupfer C"[251] and precipitated copper[252] are especially recommended; the minimum amount of copper was reported[251] to be 2g per mole of reactants.

3. A base is necessary[249] to react with the liberated hydrogen halide, and potassium carbonate is the reagent of choice[253] although a suggestion has been made[245] that a tertiary amine could act as combined solvent and base.

4. Solvents found to be useful include nitrobenzene,[254] diglyme,[255] DMSO,[256] DMF,[257] N,N-dimethylacetamide[258] and HMPT.[247b]

5. Addition of small quantities of iodine or potassium iodide has been found advantageous.[259]

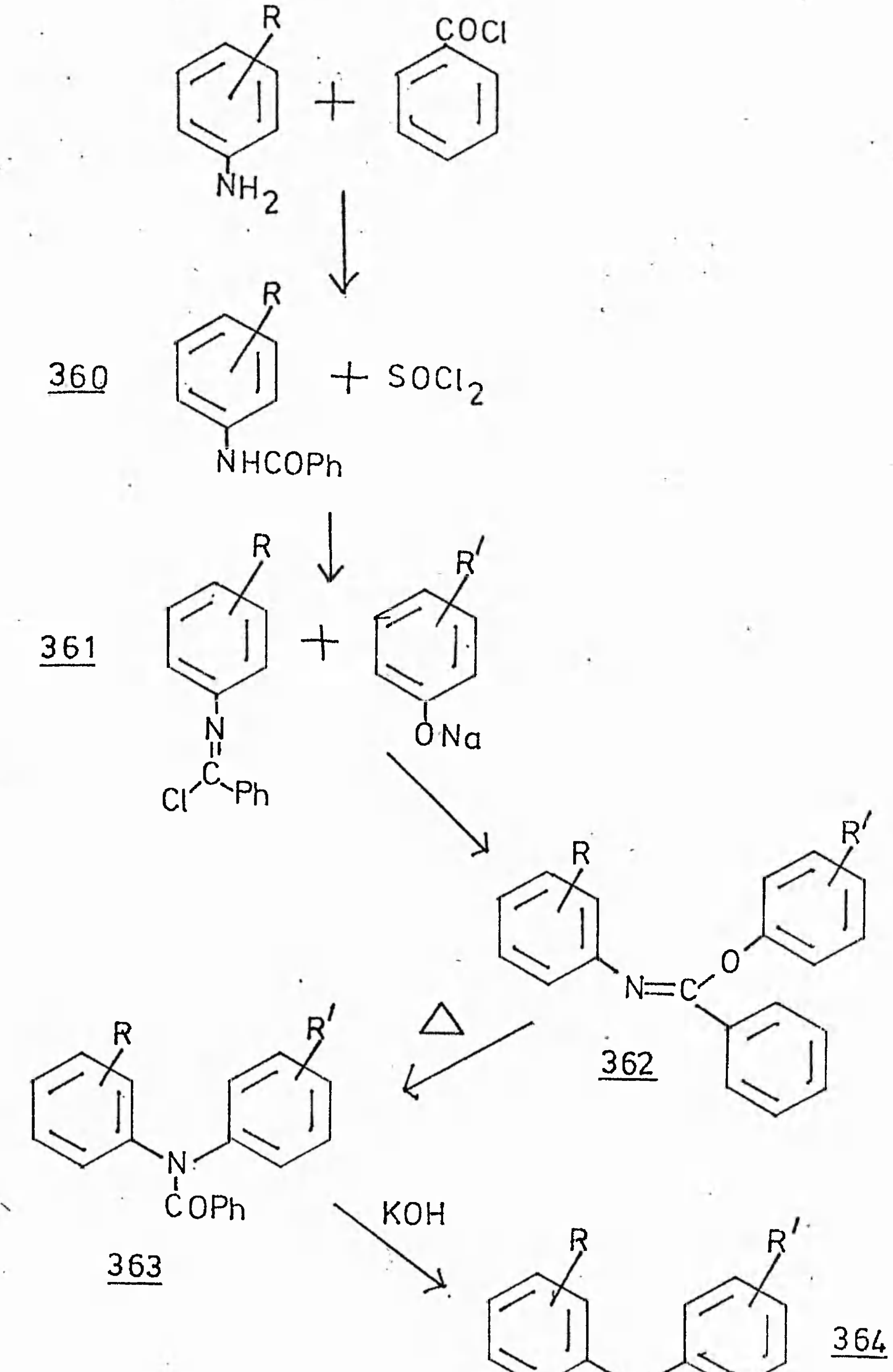
6. Presence of water, in even trace amounts, can prevent reaction.

A study[251] of the reaction between 4-methylacetanilide and bromobenzene showed that copper or a copper salt reacted with air and the amide to form an active catalytic species which was soluble in alcohol but destroyed by small amounts of water. Running the reaction without a solvent gave double the highest yield obtained with a solvent (tetrahydronaphthalene).

An alternative synthesis of diarylamines is the Chapman rearrangement.[260,248] This is the thermal rearrangement of aryl N-arylbenzimidates 357 to N-aroyldiarylamines 359 via a four-membered transition state[261] 358. The reaction can be considered[261] to be an intramolecular nucleophilic attack by the nitrogen lone-pair electrons on the ring carbon atom bonded to oxygen. In agreement with this, electron-withdrawing groups on the aryloxy-ring facilitate the reaction, while electron-donating substituents on the arylimino-ring help by increasing the electron density on the nitrogen atom. It was also found[262] that ortho substituents on the aryloxy-ring were more effective than para; this was attributed[261] to an entropy effect.

The reaction sequence from amine and phenol to diarylamine is summarised in Scheme V. Overall yields can be as high as 70% but reaction conditions are critical, particularly in the pyrolysis step, and many low yields or failures have been reported.[248,263]

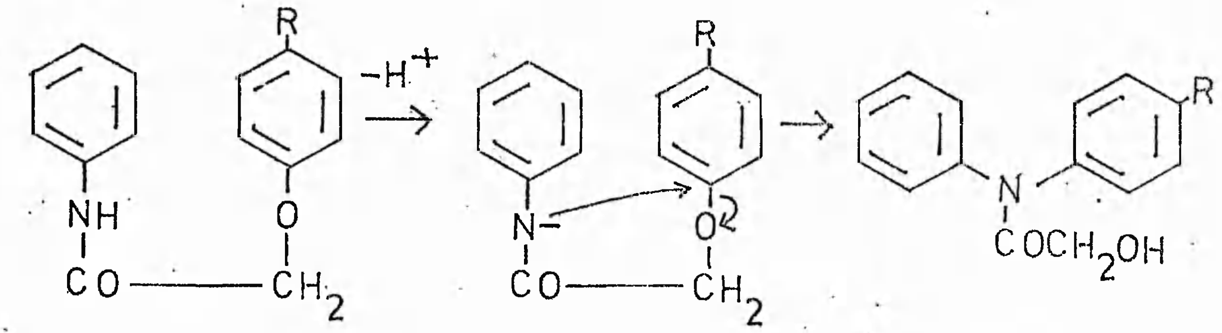
In addition to the copper-catalysed condensations and the Chapman rearrangement, several miscellaneous methods have been employed to synthesise diarylamines. Many symmetrical compounds, including diphenylamine itself, can be readily prepared by heating together an amine and its hydrochloride or by the reaction of an aniline and the corresponding phenol, but these methods are less suitable for unsymmetrically substituted compounds, which must be


R
COCl
NH2
360
R
SOCl2
NHCOPh
361
R
R′
N
C
Cl
Ph
ONa
362
R
R′
N
C
O
△
363
R
R′
N
COPh
KOH
364
R
R′
N
H

Scheme V

laboriously separated[264] from a complex mixture. 2-Amino-4'-hydroxydiphenylamine has been prepared[265] by permanganate oxidation of a mixture of 1,2-diaminobenzene and phenol. The reaction of aryl Grignard compounds with nitrobenzene[266] has given diarylamines in moderate yield, but has not been developed as a synthesis. Diphenyliodonium salts produce diarylamines on treatment with anilines.[267] Aryl halides react with the potassium salts of arylamines in liquid ammonia to afford diarylamines in good yield,[268] but, since the reaction proceeds by a benzyne intermediate, substituted aryl halides give equimolar mixtures of isomers. Other leaving groups have been used in nucleophilic displacements, e.g. a dinitrophenyl sulphonic ester[269] and a dinitrophenyl sulphone[270] have both given diarylamines on treatment with aromatic amines. In a variant[271] of the Smiles rearrangement, treatment of the amide 365 with base removed a proton to give the anion 366, which then underwent intramolecular nucleophilic displacement to yield the diphenylamine derivative 367. A recent report[242] details the electrophilic substitution by aryl azides of aromatic hydrocarbons using Lewis acid catalysts, to give diarylamines, but the products are mixtures of *ortho* and *para* isomers.

In the synthesis of 2,4'-dihydroxydiphenylamine or its N-acyl derivatives by copper-catalysed reactions the phenolic groups must be protected to prevent unwanted side-reactions such as formation of diaryl ethers. Phenol esters often decompose under the vigorous reaction conditions, and it is more common to block the hydroxyl groups by ether formation. In the present investigation three



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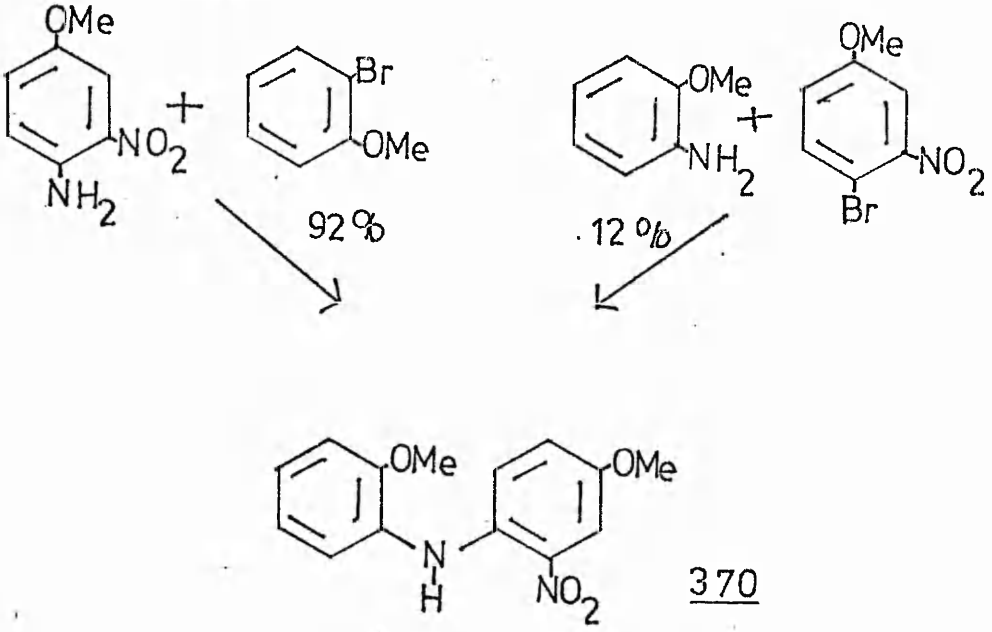

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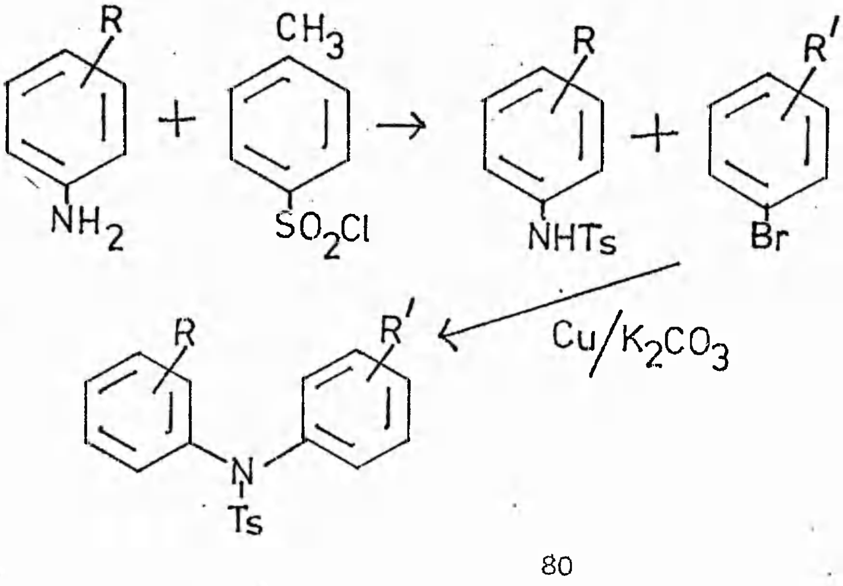


types of ether were employed, methoxy groups which can be cleaved by several methods (see later), benzyl ethers which are susceptible to hydrogenolysis or acid hydrolysis, and the methoxymethoxy group which readily decomposes to give a phenol on treatment with a trace of acid.[272] In addition other functions could be converted to phenols; a nitro substituent on reduction to the amine, followed by diazotisation and hydrolysis of the resulting diazonium salt would give a hydroxyl group. Grignard compounds can be formed from bromine substituents and can be converted to phenols either by treatment with trimethyl borate and subsequent oxidation of the resulting boronic acids by alkaline peroxide,[273] or by reaction with t-butyl perbenzoate to produce t-butyl ethers which are then easily hydrolysed by acid.[274]

2.2 Attempted Syntheses of Diarylamines by Established Methods

2.2.1 Ullmann Reactions

The initial approach to a 2,4′-dihydroxydiarylamine was to attempt an Ullmann reaction between the commercially available 4-benzyloxyaniline and the activated aryl bromide, 1-benzyloxy-2-bromo-4,6-dinitrobenzene to give a dinitro substituted 2,4′-dibenzyloxydiphenylamine. The potassium salt of 2-bromo-4,6-dinitrophenol[275] failed to form a benzyl ether when treated with benzyl chloride either in DMSO at room temperature or at 100°, or in refluxing DMF. This failure may be due to the electron-withdrawing effect of two nitro groups being sufficient to reduce the nucleophilic character of the phenoxide anion, or to the steric hindrance of the nitro group and the bromine atom *ortho* to the oxygen.

Whichever reason was important it was felt unlikely that the phenol would undergo self-reaction under Ullmann conditions and that protection was unnecessary. The bromophenol and the amine did not react on heating without a copper catalyst; in the presence of copper, both without a solvent and in DMSO solution, black tarry mixtures were obtained, chromatography of which yielded small amounts of 4,4′-dibenzyloxyazobenzene 368 (identified by spectroscopy and analysis). The same product was obtained from reactions between 4-benzyloxyaniline and 1-bromo-2-nitrobenzene in the presence of copper bronze and potassium carbonate, both without a solvent and in nitrobenzene solution. Although azobenzenes have not been reported from Ullmann reactions, they have been formed by copper-catalysed aerial oxidation of arylamines in pyridine solution.[276] The reaction of 4-benzyloxyaniline and 1-bromo-2-nitrobenzene was accomplished by heating with anhydrous sodium acetate without a catalyst, (a method similar to that[277] used for 4-methoxyaniline and 1-chloro-2-nitrobenzene) and afforded 4-benzyloxy-2′-nitrodiphenylamine 369 in 55% yield.

Further exploration of routes involving Ullmann reactions was made with the cheaper 4-methoxyaniline. A mixture of 4-methoxyaniline, 2-bromoanisole, potassium carbonate and copper bronze on heating, both alone and with the addition of a small amount of pyridine, gave a low yield of 4,4′-dimethoxyazobenzene (identified by analysis and m.p.). This product was also formed in reactions between 4-methoxyaniline and 2-bromo-5-nitroanisole both in the presence of potassium carbonate and copper bronze, and also when sodium acetate without copper was employed. It was likewise isolated when 4-methoxyaniline was heated with 2-bromo-4-nitroanisole either in the presence of potassium carbonate, copper and a small amount of pyridine, or in refluxing DMF without a catalyst. No product was obtained when the above starting materials were heated with anhydrous sodium acetate. The azobenzene was also formed in reactions between 4-methoxyaniline and 2-bromo-4,6-dinitrophenol or 2-bromo-4-nitrophenol in the presence of potassium carbonate and either copper bronze or copper (I) chloride. In all the above reactions with 4-methoxyaniline the effect of varying the temperature and duration of the heating was tried unsuccessfully and the reaction mixtures were invariably black and tarry; column chromatography was used to isolate the azobenzene in yields of 1-8%. The oxidation of the aniline to the azobenzene, although probably copper-catalysed, would seem to be facilitated by the presence of nitro groups on the aryl bromide which could also act as oxidants.

Reaction of 2-methoxyaniline and 4-bromoanisole in the presence of copper bronze and either potassium carbonate or pyridine gave no product as did similar reactions between 2-bromoaniline and 1,4-dibromobenzene; no azobenzene was detected in either case.

As the starting materials were available, it was decided to determine whether a nitro substituent in the aniline would facilitate the reaction. Accordingly a mixture of 4-methoxy-2-nitroaniline, 2-bromoanisole, potassium carbonate, copper bronze gave, on heating, 2,4'-dimethoxy-2'-nitrodiphenylamine 370 in 92% yield. Reaction of 2-methoxyaniline with 4-bromo-3-nitroanisole under identical conditions to the above gave the same product but in only 12% yield. These results are puzzling as they seem to show the reverse of the normal effect of a nitro group in the aryl bromide facilitating the reaction due to stabilisation of the Wheland intermediate, and a nitro group in the amine hindering it due to the decrease in electron density on the nitrogen atom. Another surprising result was the reaction of 2-methoxy-5-nitroaniline with 4-bromoanisole under similar conditions to produce 2,4',4''-trimethoxy-5-nitrotriphenylamine 371 (structure determined by spectroscopy and analysis) in 12% yield; no trace of corresponding diphenylamine was found.

2.2.2 Goldberg Reactions

As the above results made it seem unlikely that Ullmann reactions would furnish a practicable route to the desired diarylamine derivatives, attention was turned to Goldberg reactions. 2-Methoxyacetanilide and 4-bromoanisole, on heating in the presence of copper bronze and potassium carbonate, gave a mixture which contained the desired product together with unreacted starting materials. The unchanged acetanilide could be removed by selective hydrolysis with 10M sulphuric acid, and subsequent chromatography afforded N-2-methoxyphenyl-N-4-methoxyphenylacetamide 372 in 37% yield. Hydrolysis of the product with ethanolic potassium hydroxide gave only poor yields of a substance later shown to be

2,4′-dimethoxydiphenylamine 403 by comparison with an authentic sample (p. 93).

Attempts to react 4-bromoacetanilide with 2-bromoanisole, and 4-nitroacetanilide with 1-bromo-2-nitrobenzene under a variety of conditions, were unsuccessful; a surprising result in the latter instance as 4-nitroacetanilide has been reported[278] to react in good yield with bromobenzene.

Since 1-benzyloxy-2-bromobenzene decomposes on heating,[279] it cannot be used in a Goldberg reaction with 4-benzyloxyacetanilide to produce N-acetyl-2,4′-dibenzyloxydiphenylamine, and the less readily available 2-benzyloxyacetanilide was therefore prepared. 2-Aminophenol was selectively N-acetylated by the method of Fiers-David and Kuster[280] and O-benzylated as described elsewhere;[281] 4-bromophenol was benzylated in dry acetone in the presence of potassium carbonate to give 1-benzyloxy-4-bromobenzene in 95% yield. The reaction of the aryl bromide with the acetanilide was carried out under a variety of conditions with unsatisfactory results; extensive decomposition took place and repeated chromatography gave only trace amounts of a material with an R_f different to that of starting material and with the correct infrared absorptions.

2.2.3 Chapman Rearrangements

The initial aim was to prepare the benzimidate 362 (R = 4-$PhCH_2O$, R^1 = 2-$PhCH_2O$) by reaction of the known[282] N-4-benzyloxyphenylbenzimidoyl chloride with the sodium salt of 2-benzyloxphenol, and to rearrange this to the N-N-diphenylbenzamide 363 ($R = 4\text{-}PhCH_2O$, $R^1 = 2\text{-}PhCH_2O$). Treatment of N-4-benzyloxyphenylbenzamide with thionyl chloride furnished the benzimidoyl chloride in good yield, but numerous attempts to induce it to react with sodium 2-benzyloxyphenoxide under the standard conditions[248] (see Experimental) were unsuccessful, as were attempts using solvents which favour nucleophilic displacements (DMSO and DMF) and an attempt employing the free phenol in pyridine, (a method reported[269] as an improvement in difficult cases). Heating the reaction mixtures did not promote reaction and in all cases N-4-benzyloxyphenylbenzamide was quantitatively recovered on quenching the reaction with water; it would seem that the anion of 2-benzyloxyphenol was too sterically hindered to react. N-4-benzyloxyphenylbenzimidoyl chloride did, however, react with the sodium salts of 2-methoxyphenol and 2-nitrophenol to produce respectively 2-methoxyphenyl N-4-benzyloxyphenylbenzimidate 373 in 32% yield, and 2-nitrophenyl N-4-benzyloxyphenylbenzimidate 374 in 55% yield. An attempt to use the monosodium salt of 1,2-dihydroxybenzene in a reaction with the above benzimidoyl chloride produced, after chromatography, a small amount of homogeneous, amorphous material with the expected infrared spectrum; an attempt to characterise this as the O-ethoxycarbonyl derivative likewise gave amorphous material.

The desired 2,4′-dibenzyloxybenzimidate was later prepared by the alternative route. N-2-Benzyloxyphenylbenzamide 375 was prepared in a similar manner to 2-benzyloxyacetanilide, and on treatment with thionyl chloride furnished the benzimidoyl chloride as an oil which could not be crystallised, (many benzimidoyl chlorides are liquids at room temperature[248]). Reaction with the sodium salt of 4-benzyloxyphenol under the standard conditions afforded 4-benzyloxyphenyl N-2-benzyloxyphenylbenzimidate 376 in 70% yield. In one experiment the only product isolated (in moderate yield) was identified by analysis, n.m.r. spectroscopy and m.p., as 1,4-dibenzyloxybenzene, which was possibly formed by reaction between the benzimidoyl chloride and thionyl chloride still present to give benzyl chloride which then benzylated the 4-benzyloxyphenoxide.

N-4-Methoxyphenylbenzimidoyl chloride and the sodium salt of 2-methoxyphenol afforded 2-methoxyphenyl N-4-methoxyphenylbenzimidate 377 in 93% yield, while reaction of N-2-bromophenylbenzimidoyl chloride[283] with the sodium salt of 4-bromophenol gave material which t.l.c. showed to be a complex mixture; purification was therefore not attempted. This was disappointing considering that the 2,2′-dibromobenzimidate had been prepared[283] in good yield.

The optimum duration and temperature of the pyrolysis necessary to rearrange benzimidates varies widely[248] and can only be determined by trial and error. The dimethoxybenzimidate 377 gave a mixture of three components, none of which could be obtained crystalline although it is likely that one component was the diarylbenzamide and another the free diphenylamine (see Experimental). The optimum conditions for rearrangement of the 4-benzyloxy-2′-nitrobenzimidate 374 were found to be 200° for 20 minutes when an

80% yield of N-4-benzyloxyphenyl-N-2-nitrophenylbenzamide 378 was obtained. Rearrangement of the 2,4′-dibenzyloxybenzimidate 376 under a variety of conditions gave a mixture of five components, a minor one of which was unchanged benzimidate. Two others, from their chromatographic and spectral properties, were assumed to be the diarylbenzamide and the free diphenylamine although they could not be obtained pure. Attempts at purification by sublimation under reduced pressure led to decomposition and production of 1,2-dihydroxybenzene. The results from the rearrangements were disappointing except for the 2-nitrophenyl case which was expected to react well due to the activating effect of the electron-withdrawing substituent; however, many other cases of benzimidates failing to rearrange have been reported.[248]

2.3 Synthesis of N,N-diaryltoluene-4-sulphonamides

None of the above reactions promised to provide a good preparative route to the desired 2,4′-dihydroxydiphenylamine derivatives, with the possible exceptions of compounds 372 and 378. Considering this and also the fact that an N-arylsulphonyl function would be especially suitable as a blocking group during the oxidation to the spirodienone, it was thought possible that N-arylarylsulphonamides might react with aryl bromides in a Goldberg-type reaction. Primary arylsulphonamides are known to undergo nucleophilic reactions with activated aryl halides, the reactions being catalysed by copper, copper (I) or copper (II) salts;[284-7] N-arylarylsulphonamides react with alkyl halides without catalysis by copper.[288]

Accordingly, N-4-methoxyphenyltoluene-4-sulphonamide was heated with 2-bromoanisole, potassium carbonate and copper bronze to give N-2-methoxyphenyl-N-4-methoxyphenyltoluene-4-sulphonamide 379 in 60% yield. The success of this reaction and also the fact that unreacted secondary sulphonamide was easily removed from the reaction mixture by extraction with alkali, led to further investigation of the scope of the synthesis. A wide range of substituted N-aryltoluene-4-sulphonamides was treated with aryl bromides, bearing both electron-withdrawing and electron-donating substituents, under a variety of conditions, and the optimum time and temperature for the reaction determined. It was found that the best yields were obtained by heating at 180° for 24 hours (less in the case of aryl bromides bearing nitro substituents) without a solvent but with the addition of a 20% excess of aryl bromide. The catalytic effect of copper bronze and precipitated copper[252] was equal but greater than that of copper (I) or copper (II) salts; the addition of iodine or potassium iodide did not increase the yield. When the reaction is carried out on a large scale with an activated aryl bromide it can become exothermic (particularly in the first hour) and care is needed lest the flask overflow due to the frothing produced by evolution of carbon dioxide.

The yields of tertiary sulphonamides obtained are shown in Table I. It would be expected that the highest yields would result from the presence of electron-withdrawing substituents in the aryl bromide, and electron-donating ones in the N-arylsulphonamide which would increase the nucleophilicity of the nitrogen atom. This is

Table I

Reaction of sulphonamides $RC_6H_4\cdot NH\cdot SO_2\cdot C_6H_4Me$-4 with bromides $R'C_6H_4Br$

No.	R	R'	Yield of N,N-diarylsulphonamide (%)	Yield by modified method (%)
379	4-MeO	2-MeO	63	(67)
379	2-MeO	4-MeO	84	(92)
380	4-MeO	4-MeO	82	
381	4-MeO	4-Me	45	
381	4-Me	4-MeO	29	(73)
382	4-MeO	H	70	
382	H	4-MeO	14	
383	4-Me	H	7	(44)
383	H	4-Me	12	
384	4-Me	4-Me	9	
385	2-MeO	4-Ph	85	
386	2-MeO	4-Cl	20	
387	2-MeO	4-MeO,2-Me	76	
388	3,4-diMe	2-MeO	9	
389	4-$PhCH_2O$	4-MeO	67	
390	2-$PhCH_2O$	4-$PhCH_2O$	76	(61)
391	H	4-NO_2	91	
392	4-Me	4-NO_2	64	
393	4-MeO	4-NO_2	84	
394	3-MeO	4-NO_2	86	
395	4-$PhCH_2O$	4-NO_2	74	
396	4-$PhCH_2O$	2-NO_2	98	
397	3,4-diMe	2-NO_2	61	
	3-MeO	4-MeO	0	(0)
	3-MeO	H	0	
	H	H	9	

found to be so, but also alkoxy and phenyl groups in the aryl bromide do not lower the yields; it is probable that the substituent in the sulphonamide is more important, as shown by the low yields obtained when R is H or Me and R$^{\prime}$ is other than nitro. In agreement with this is the different between reactions where R = 4-methoxy, (compounds 380 and 382) and similar reactions when R = 3-methoxy; the failures in the latter case are probably due to the absence of the activating mesomeric effect of the alkoxy group. The 4-nitrobenzesulphonamides of aniline and 4-methoxyaniline were prepared and treated with 4-bromoanisole under the above conditions to give N-4-methoxyphenyl-N-phenyl-4-nitrobenzenesulphonamide 398 and N,N-bis-(4-methoxyphenyl)-4-nitrobenzenesulphonamide 399 in 12 and 51% yields respectively. The nitro group in the sulphonamide moiety would be expected to reduce the electron density on the nitrogen atom but only by an inductive effect, not a mesomeric one, and this may explain why, although the yields were less than those in the corresponding 4-toluenesulphonyl cases 382 and 380 viz 14 and 82%, they were not markedly so.

N-4-Nitrophenyltoluene-4-sulphonamide was treated with aryl bromides under the above conditions to give mixtures which were shown by t.l.c. to be free from tertiary sulphonamide. Chromatography afforded small amounts of products shown by analysis and n.m.r. spectroscopy to be triarylamines. Reaction with bromobenzene gave 4-nitrotriphenylamine[289] 400 in 7% yield, while from 4-bromotoluene was obtained 4,4$^{\prime}$-dimethyl-4$^{\prime\prime}$-nitrotriphenylamine 401 in 6% yield, and from 1-bromo-4-nitrobenzene was isolated

4,4′,4″-trinitrotriphenylamine 402 in 4% yield. No product was obtained from reactions between N-4-nitrophenyltoluene-4-sulphonamide and 4-bromoanisole, or between N-2-nitrophenyltoluene-4-sulphonamide and either bromobenzene or 4-bromotoluene. To elucidate the mechanism of formation of the triarylamines, N-4-nitrophenyl-N-phenyltoluene-4-sulphonamide 391 was heated with bromobenzene, potassium carbonate and copper to give 4-nitrotriphenylamine 400 in low yield. The mechanism of this reaction is obscure but possibilities include the oxidative formation of a complex between the tertiary sulphonamide and copper leading to generation of a negative charge on the nitrogen atom, and a free radical mechanism. Further experiments which might have distinguished between these possibilities were not attempted due to insufficient time. The fact that triarylamines were not formed when a nitro-substituted tertiary sulphonamide was formed from a bromonitrobenzene (compounds 391-397), is probably explained by the fact that the yields in these reactions were sufficiently high to leave no great excess of aryl bromide present.

While this work was in progress, Bacon and Karim[290] reported that if N-phenylbenzenesulphonamide was converted into its sodium salt with sodium hydride in N,N-dimethylacetamide, and the product was then heated with bromobenzene and copper (I) iodide in the same solvent, a poor yield of N,N-diphenylbenzenesulphonamide was obtained. Several of the N,N-diaryltoluene-4-sulphonamides were prepared by this alternative route and the yields are shown in parentheses in Table I. A distinct improvement in reactions using the toluene-4-sulphonamide of 4-toluidine was noticed; this is probably due to reduction of the extensive thermal decomposition which occurred in the Goldberg-type procedure. The latter reaction, however, is cheaper and simpler to carry out and is to be preferred in the majority of cases.

The tertiary sulphonamides, now readily available, should be useful precursors for the preparation of unsymmetrically substituted diphenylamines, provided that the sulphonamide function can be removed. Diphenylamine has been obtained quantitatively from its toluene-4-sulphonyl derivative by treatment with sodium 3-methylbut-1-oxide in refluxing 3-methylbut-1-ol,[291] and in 88% yield by reaction of the sulphonamide with tetramethylammonium, generated at a mercury cathode.[292] Secondary N-arylarylsulphonamides are cleaved by 40% sulphuric acid in acetic acid.[293] The 3-methylbutoxide method was applied to compound <u>379</u> and <u>2,4′-dimethoxydiphenylamine</u> <u>403</u> was obtained in 68% yield, which is equivalent to an overall yield of 60% from the methoxyaniline and bromoanisole. A similar attempt to prepare 2,4′-dibenzyloxydiphenylamine was unsuccessful, probably due to the lability of the benzyl ethers; however, a small amount of impure material with the correct spectral properties was isolated.

In order to produce authentic material to compare with the products of earlier experiments, the acylation of 2,4′-dimethoxydiphenylamine <u>403</u> was investigated. Compound <u>403</u> did not react with acetic anhydride, acetyl chloride or benzoyl chloride even on heating in pyridine. When the method of Maki et al[294] which employed catalysis by anhydrous zinc chloride was used, acetic anhydride gave a 57% yield of the N-acetyl derivative identical to compound 372, while benzoyl chloride gave a mixture, one of whose components had the same R_f as material obtained from the Chapman rearrangement of the 2,4′-dimethoxybenzimidate 377.

2.4 Approaches to N-2-hydroxyphenyl-N-4-hydroxyphenyltoluene-4-sulphonamide

2.4.1 Demethylation Experiments

The demethylation of the tertiary sulphonamide 379 was the simplest route to the desired diphenol, and it was expected to be fairly straightforward. However, treatment of 379 with constant-boiling hydriodic acid under reflux in the presence of red phosphorus,[295] or hydrogen bromide in refluxing acetic acid,[296] or anhydrous aluminium chloride in boiling benzene,[297] or pyridine hydrochloride at either 180° or 250°,[298] or finally with boron tribromide in dichloromethane at 0°,[299] all failed to give the desired product, producing instead an intractable black solid (for properties of which see Experimental).

Similar attempts at demethylating the diphenylamine 403 with hydrogen bromide in acetic acid, aluminium chloride or pyridine hydrochloride were also unsuccessful. A recent report[300] details a new reagent, sodium 4-methylbenzenethiolate in toluene with HMPT added, which cleaves both methyl and benzyl ethers under mild conditions and in excellent yields; it would have been interesting to have used this reagent in the present cases.

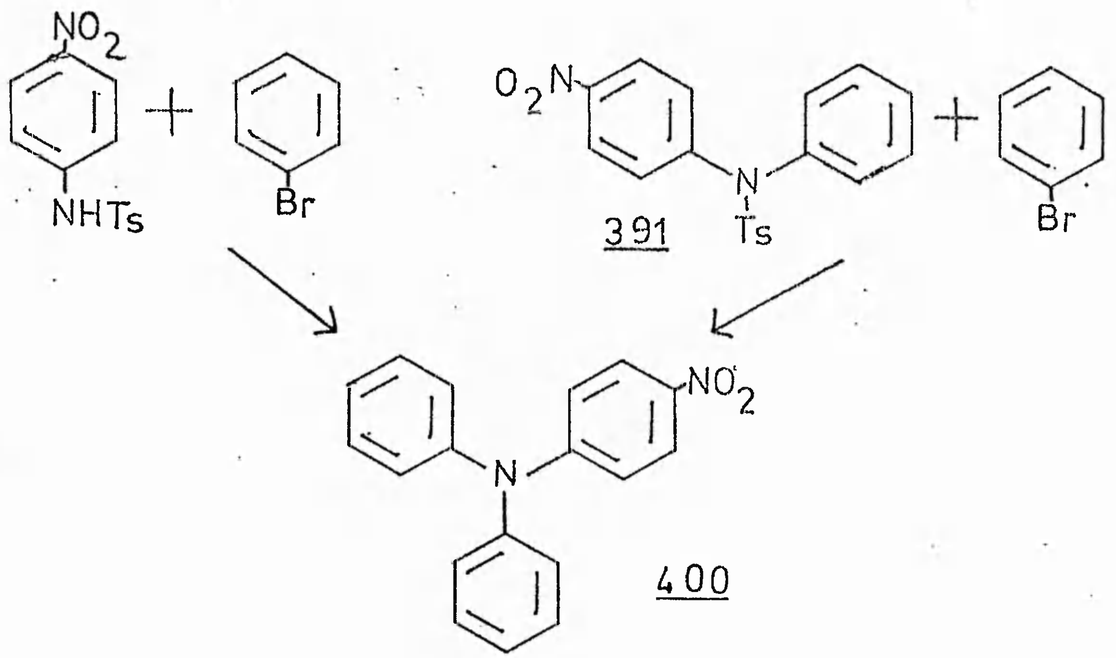
2.4.2 Methoxymethyl Ethers

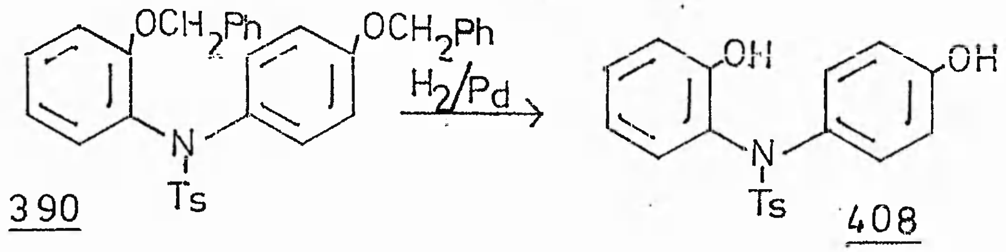
This protecting group[272] is very easily removed with dilute acid and was thought to be stable to conditions in the Goldberg-type reaction. The sodium salt of 2-nitrophenol was treated with chloromethyl methyl ether in benzene to produce a 94% yield of 1-methoxymethoxy-2-nitrobenzene 404, which could then be reduced to 2-methoxymethoxyaniline by sodium dithionite in refluxing aqueous ethanol or, in better yield, by catalytic hydrogenation over palladium. The aniline was unstable and although distillation gave a clean product, it rapidly darkened even on storing in the dark under nitrogen; for this reason it was converted immediately to N-2-methoxymethoxyphenyltoluene-4-sulphonamide 405. Previous attempts to prepare the aniline by methoxymethylation of 2-hydroxyacetanilide to be followed by hydrolysis, had given no product, while a similar reaction of N-2-hydroxyphenyltoluene-4-sulphonamide had formed the N-methoxymethyl compound which was not further purified. 1-Bromo-4-methoxymethoxybenzene 406 was prepared in 46% yield from 4-bromophenol.

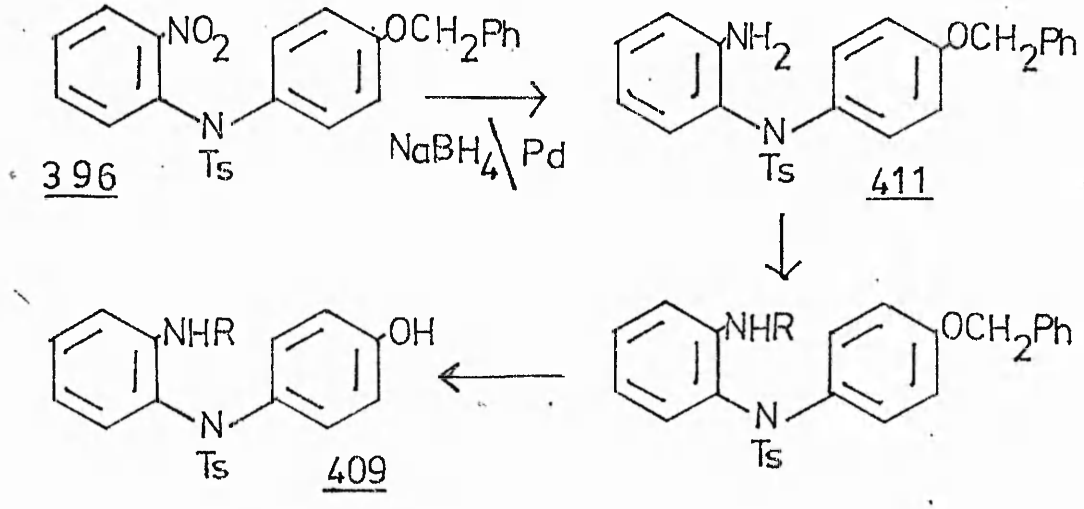
Reactions of 405 with 406 were attempted under a wide range of conditions but only mixtures containing large amounts of tar were obtained; repeated chromatography yielded a small amount of amorphous material which had the n.m.r. spectrum expected for the tertiary sulphonamide but it could not be further purified. Reaction under the conditions of Bacon and Karim[290] was likewise unsuccessful.

2.4.3 Benzyl Ethers

Because of the thermal instability of 1-benzyloxy-2-bromobenzene







R= COPh, CH_2Ph, Ts, 2,4-di-NO_2Ph

it could not be used in a Goldberg-type reaction with N-4-benzyloxyphenyltoluene-4-sulphonamide and it was therefore necessary to prepare N-2-benzyloxyphenyltoluene-4-sulphonamide. An attempt to benzylate N-2-hydroxyphenyltoluene-4-sulphonamide[301] gave only N-benzyl-N-2-hydroxyphenyltoluene-4-sulphonamide 407 in 76% yield. However, 2-benzyloxyacetanilide was hydrolysed[281] to 2-benzyloxyaniline and this was converted to the toluene-4-sulphonamide. This secondary sulphonamide was unique amongst those used, in being insoluble in all alkalis (sodium, potassium, lithium and tetraalkylammonium hydroxides). The Goldberg reaction, however, with 1-benzyloxy-4-bromobenzene gave a sufficiently high yield of tertiary sulphonamide to enable the product to be easily isolated. The 2,4'-dibenzyloxysulphonamide 390 was hydrogenolysed over palladium to afford N-2-hydroxyphenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 408 in 80% yield.

2.5 Preparation of Other Substrates for Oxidation

Owing to the results obtained from various oxidation experiments (see Chapter 3), it became desirable to prepare, as substrates for oxidation, compounds of the type 409 where R is hydrogen, alkyl, aryl or acyl, and also the secondary toluene-4-sulphonyl derivatives of 2-amino-4'-hydroxydiphenylamine and 2-amino-4'-diphenyl ether.

N-4-Benzyloxyphenyl-N-2-nitrophenyltoluene-4-sulphonamide 396 was catalytically hydrogenated to afford N-2-aminophenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 410 in 80% yield. A useful method of selectively reducing aromatic nitro groups in the presence of benzyl ethers has been reported,[302,303] which used sodium borohydride catalysed by palladised charcoal; in the present case this method gave much better results than the use of sodium dithionite. The nitrosulphonamide 396 furnished N-2-aminophenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 411 in 90% yield. This compound gave N-2-benzamidophenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 412 and N-4-benzyloxyphenyl-N-2-(toluene-4-sulphonamido)-phenyltoluene-4-sulphonamide 413 in 67 and 97% yields respectively on treatment with the appropriate acyl chloride. Reaction of 411 with benzyl chloride afforded N-2-benzylaminophenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 414 in 68% yield. Compound 411 did not react with 1-fluoro-2,4-dinitrobenzene on refluxing in DMF in the presence of potassium carbonate, but in an Ullmann reaction catalysed by copper bronze it gave N-4-benzyloxyphenyl-N-2-(2',4'-dinitrophenylamino)-phenyltoluene-4-sulphonamide 415 in 45% yield. Compound 411 failed to react with 2-bromopropane on refluxing with potassium carbonate in acetone, and, on treatment with methanesulphonyl chloride, a three component mixture was obtained which was not further purified.

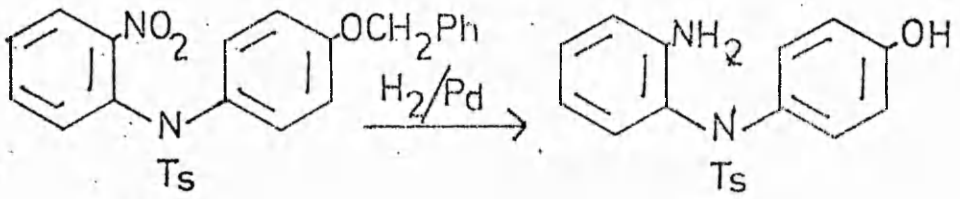
The cleavage of the benzyl ethers in compounds 412-415 presented a problem as the results which were being obtained with catalytic hydrogenolysis were erratic. A useful method has been reported[303] in which trifluoroacetic acid (TFA) at room temperature converts benzyl ethers to phenols in good yields without hydrolysing the amide group. Use of this reagent debenzylated compound 412 to N-2-benzamidophenyl-N-4-hydroxyphenyltoluene-4-

sulphonamide 416 in 73% yield, and gave N-2-(2',4'-dinitrophenylamino)-phenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 419 in 70% yield from 415. Treatment of compounds 413 and 414 with TFA gave mixtures containing several components, but they were successfully debenzylated by prolonged heating with ethanolic hydrochloric acid to furnish N-4-hydroxyphenyl-N-2-(toluene-4-sulphonamido)-phenyltoluene-4-sulphonamide 417 in 71% and N-2-benzylaminophenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 418 in 76% yields respectively.

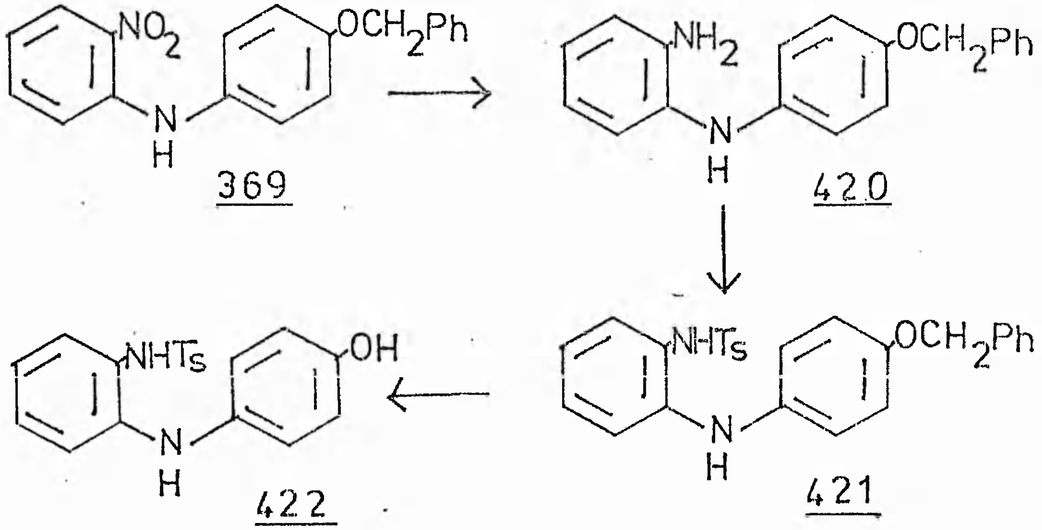
4-Benzyloxy-2'-nitrodiphenylamine 369 on reduction with borohydride and palladium afforded a good yield of 2-amino-4'-benzyloxydiphenylamine 420 which was selectively tosylated in the primary amino group to produce 4-benzyloxy-2'-(toluene-4-sulphonamido)-diphenylamine 421. This compound was best debenzylated by catalytic hydrogenolysis to form 4-hydroxy-2'-(toluene-4-sulphonamido)-diphenylamine 422 in 74% yield.

4-Benzyloxyphenol was reacted with 1-bromo-2-nitrobenzene by a modification of Bacon and Karim's procedure to give a 41% yield of 4-benzyloxy-2'-nitrodiphenyl ether[304] 423 which, on reduction, produced 2-amino-4'-benzyloxydiphenyl ether 424 almost quantitatively. This compound was converted to 4-benzyloxy-2'-(toluene-4-sulphonamido)-diphenyl ether 425 and debenzylated with ethanolic hydrochloric acid to afford 4-hydroxy-2'-(toluene-4-sulphonamido)-diphenyl ether 426 in 93% yield.

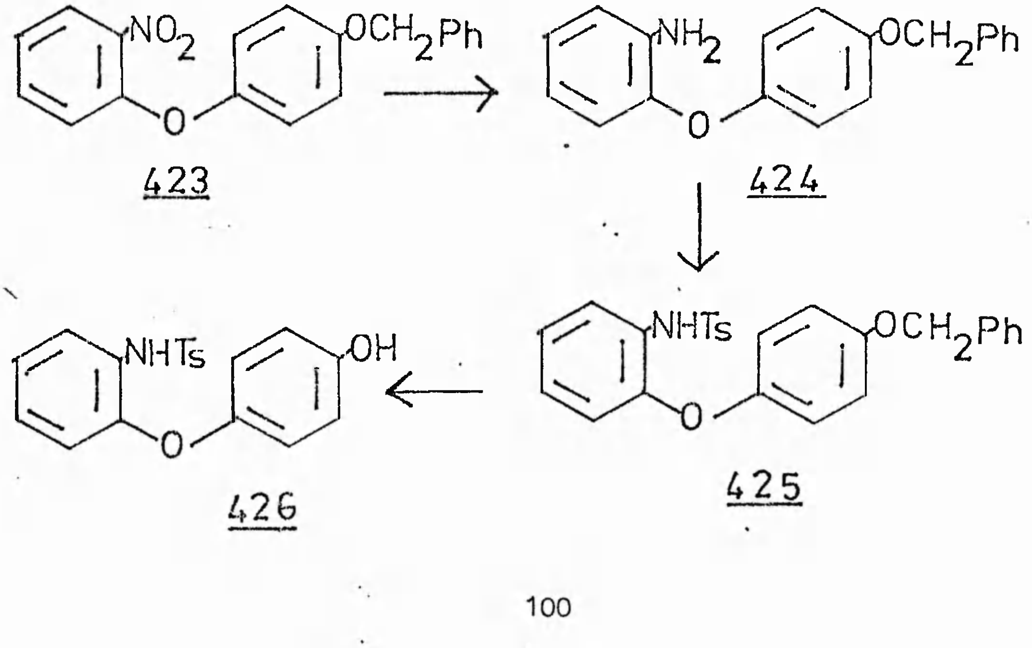
An alternative method of preparing N-4-benzyloxyphenyl-N-2-nitrophenylbenzamide 378, (first prepared by the rather laborious Chapman rearrangement route) was developed in which N-4-benzyloxy-



396 410



369 420 421 422



423 424 425 426

phenylbenzamide was heated with 1-bromo-2-nitrobenzene in the presence of potassium carbonate, copper bronze and HMPT to afford 378 in 25% yield. This preparation is a variant of the Goldberg reaction which has rarely, if ever, been used. Catalytic hydrogenation of 378 gave intractable mixtures, the infrared spectra of which showed the absence of carbonyl absorptions. Reduction of 378 by borohydride and palladium gave N-2-aminophenyl-N-4-benzyloxyphenylbenzamide 427 but only in poor yield; the product was unstable unless pure and tended to decompose during recrystallisation. Reaction of 427 with toluene-4-sulphonyl chloride formed N-4-benzyloxyphenyl-N-2-(toluene-4-sulphonamide)-phenylbenzamide 428 in 80% yield, but the difficulties in the reduction step meant that only small quantities of 428 were available for debenzylation. Initial attempts with TFA and ethanolic hydrochloric acid gave mixtures and lack of time led to this route being abandoned.

2,4'-Dihydroxybenzanilide 429 was prepared in high yield from phenyl salicylate and 4-aminophenol according to the literature method.[305]

CHAPTER THREE

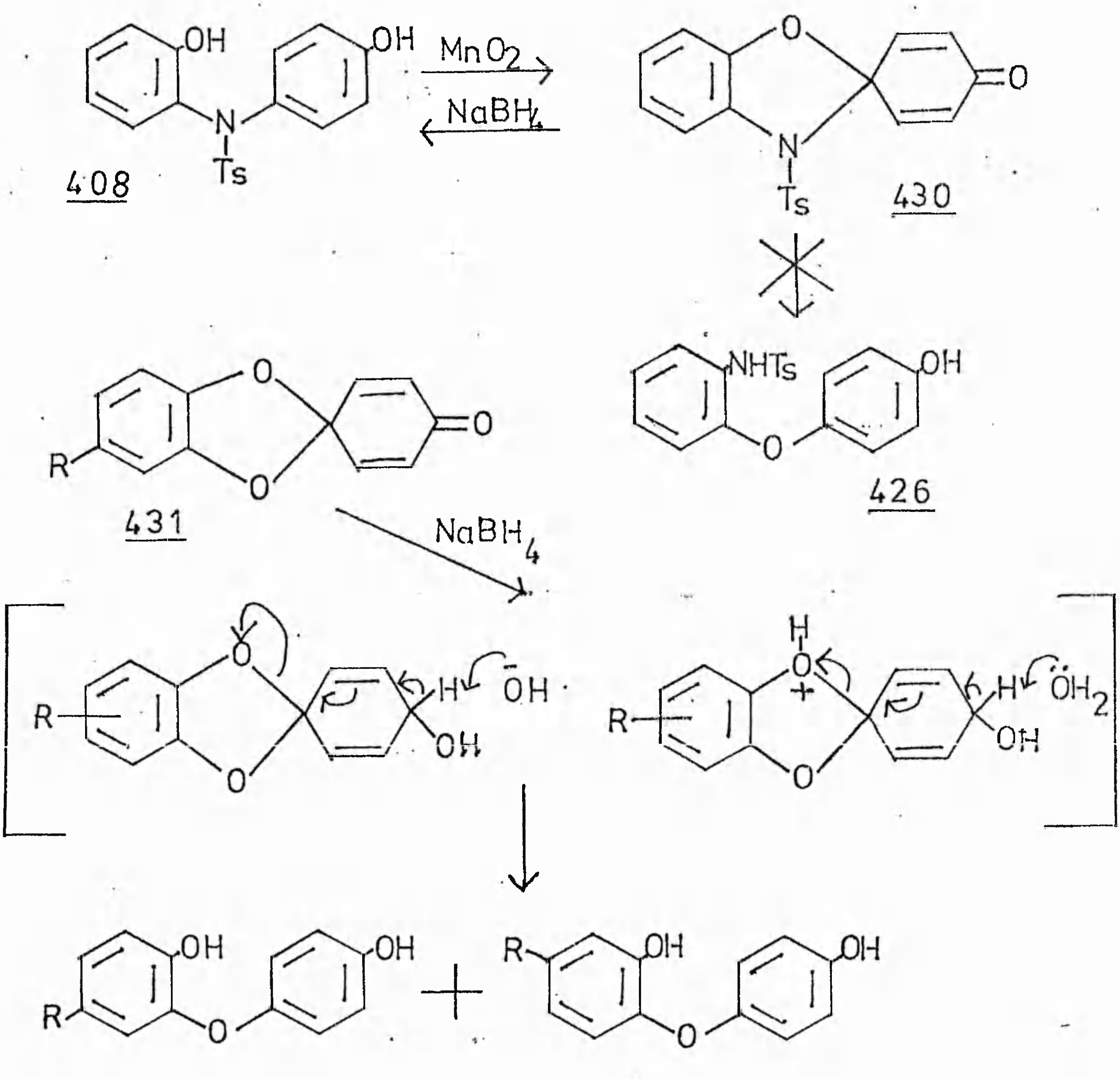
OXIDATION EXPERIMENTS AND REACTIONS OF SPIRODIENONES

3.1 Introduction

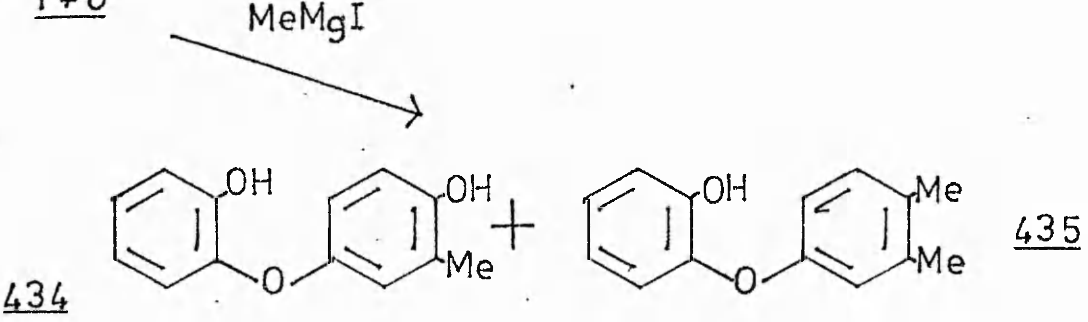
The use of phenol oxidative coupling as a route to spirocyclohexadienones has been discussed in Chapter One. The range of oxidants which have been used is wide, but potassium ferricyanide - either in alkaline solution or as part of a two-phase system - has been the most popular. Other frequently used oxidants are lead dioxide, manganese dioxide, manganese (III) compounds, silver oxide and DDQ. Vanadium oxytrihalides are more recently developed reagents, but have been particularly used in the oxidation of non-phenolic or monophenolic compounds rather than diphenols. Considering the success achieved in the oxidation of dihydroxydiaryl ethers[116] using manganese dioxide, this reagent was chosen first in the present case. A recent comprehensive review[306] of manganese dioxide as an oxidant in organic chemistry has appeared.

3.2 Synthesis and Reactions of the Benzoxazole Spirodienone 430

N-2-Hydroxyphenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 408, when heated under reflux in benzene with ten times its weight of active manganese dioxide (prepared according to the method of Franck and Blaschke[307]), afforded the desired product, N-(toluene-4-sulphonyl)-2,3-dihydro-1,3-benz[d]oxazole-2-spiro-1′-cyclohexa-2′,5′-dien-4′-one 430 in 46% yield. The success of this reaction was dependant on the type of manganese dioxide used; commercial material or that prepared by other methods[308,309] gave virtually no product. The dienone 430 was stable and highly crystalline. Its infrared



Scheme VI



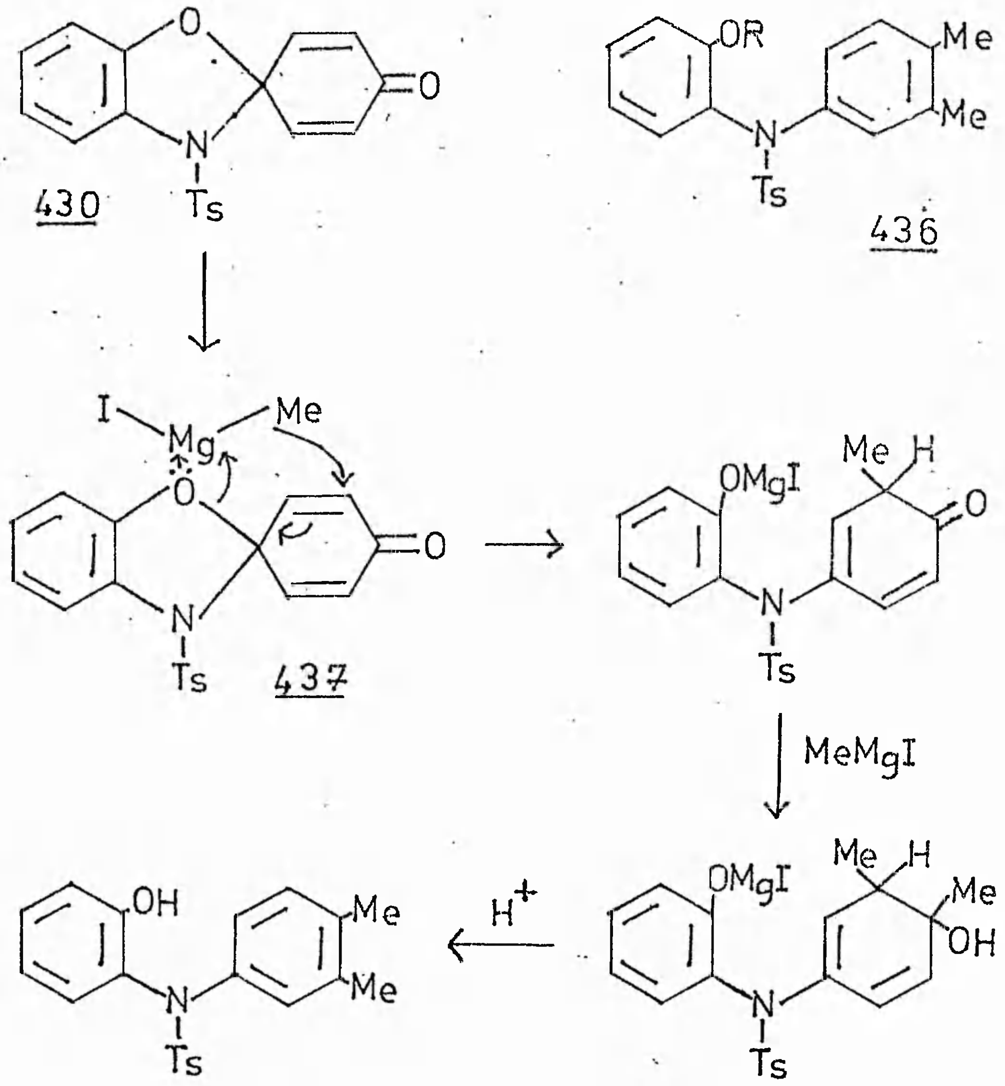
spectrum showed three bands at 1682, 1645 and 1613 cm^{-1}, which coincide with those reported[310,311] to be the characteristic stretching absorptions of cyclohexa-2,5-dienones (1640-1680, 1615-1650 and 1590-1620 cm^{-1}). In the n.m.r. spectrum the four vinylic protons appear as a quartet between 3.38 and 3.96τ; this is a typical A_2B_2 pattern with J_{AB}= 10.2 Hz, which again accords with the literature[310,312] value of $J_{AB} \approx 9.0$-10.5 Hz.

Three attempts were made to induce the dienone 430 to undergo the dienone-phenol rearrangement. The first employed the standard reagent (see Section 1.2), acetic anhydride containing a catalytic quantity of concentrated sulphuric acid at room temperature, but the reaction mixture was shown by t.l.c. to contain five components, of which a minor one corresponded to starting material. Both column and preparative thin layer chromatography failed to afford any of these components in a pure state. The second attempt used acetic anhydride containing a little boron trifluoride etherate, a method used by Hewitt,[24] but starting material was quantitatively recovered. The third utilised 100% phosphoric acid at 170°, the severe conditions which were necessary[132] to rearrange the spirodienone lactams 216, but in the present case only decomposition occurred.

An attempt to reduce the spirodienone 430 to the corresponding dienol with borohydride led to the quantitative recovery of the diphenol 408; similar results have been obtained in this laboratory[313] when the dienones 431 (R = H, Me or NO_2) were reduced. In the latter case two possible mechanisms could operate (Scheme VI), the first involving a protonation step on the heterocyclic oxygen of the dienol, and the second proceeding via removal of a proton from the alcohol carbon. Evidence was obtained for the latter path by analysis of the ratios of the products when R = Me (30% of 432, 70% of 433) and when R = NO_2 (75% of 432, 25% of 433). When an electron-withdrawing substituent is present, ring-opening occurs preferentially at the para-oxygen atom, suggesting that formation of a phenoxide anion (which is resonance stabilised) is more important than protonation of the less basic oxygen atom. The results obtained when R = Me also support formation of the anion since protonation, if it occurred, would be more likely at the para-oxygen.

In the case of 430 the second mechanism could explain why only 408 and none of the isomeric diphenyl ether 426, was produced, as a phenoxide anion is likely to be more readily formed than the alternative secondary sulphonamide anion.

Following results[314] obtained in this laboratory, which showed that the dienone[116] 178 reacted with a Grignard reagent to yield a mixture of two products, 434 and 435, it was of interest to determine whether compound 430 would behave in a similar fashion. Accordingly, 430 was treated with an excess of methylmagnesium iodide and subsequent acid hydrolysis gave a quantitative yield of a substance which n.m.r. and infrared spectroscopy suggested was the monophenol 436 (R = H). This product was treated with dimethyl sulphate in alkali and afforded

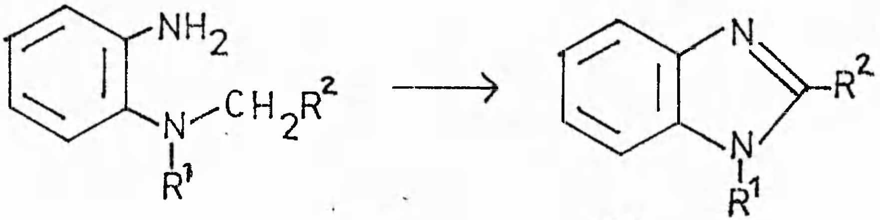


430

436

437

Scheme VII



438

439

436 (R = Me) in 70% yield after purification by preparative t.l.c. The structure of this latter compound was confirmed by an unambiguous synthesis; 2-bromoanisole, N-3,4-dimethylphenyltoluene-4-sulphonamide, potassium carbonate and copper were heated together to furnish a low yield of N-2-methoxyphenyl-N-3,4-dimethylphenyltoluene-4-sulphonamide 388, identical to the material 436 (R = Me) from the Grignard reaction, by t.l.c. in two systems, infrared and n.m.r. spectroscopy and mixed m.p.

The formation of this unusual product is thought to proceed by initial attack by the Grignard at the 3-position of the α,β-unsaturated carbonyl system, followed by further 1,2-addition and dehydration of the resulting carbinol. The driving force for the initial attack at the abnormal position may be the formation of a cyclic intermediate 437, with the magnesium co-ordinating to the heterocyclic oxygen atom, (a similar intermediate has been proposed[315] in another reaction). The suggested mechanism is shown in Scheme VII. It is interesting that in the case of the dioxygen dienone 178, products of the addition of both one and two molecules of Grignard were isolated but in the present case there was quantitative addition of two molecules. The only other case of 1,3-addition of a Grignard to an α,β-unsaturated ketone is the formation[316] of a small amount of ethyl 2-t-butylhydrocinnamate from a reaction between ethyl cinnamate and t-butylmagnesium chloride; a free radical mechanism was proposed for this reaction.

3.3 Oxidation of Compounds Containing Secondary Nitrogen Functions

Although cyclisations induced by oxidative coupling of phenols are well established, less work has been done on analogous reactions of nitrogen-containing functions. A series of substituted 1,2-diaminobenzenes 438 was oxidised[317] to the corresponding benzimidazoles 439, while the same reagent converted[318] 2,2'-diaminobiphenyl to dibenzopyridazine 440, and 2,2'-diaminodiphenyl ether to[319] 4,4'-azophenoxazine 441. Similar oxidation produced[320] the phenazine derivative 442 from 2-aminodiphenylamine while the use[321] of either manganese (III) acetate or potassium persulphate cyclised a series of 2-aminobenzophenones to afford the corresponding 9-acridanones, (the cyclisation proceeded best to a position *ortho* or *para* to an oxygen function in the other ring). Persulphate oxidation of 2-alkylamino-1,4-naphthoquinones led[322] to formation of carbazolediquinones 443; primary amino groups did not give nitrogen-coupled products.

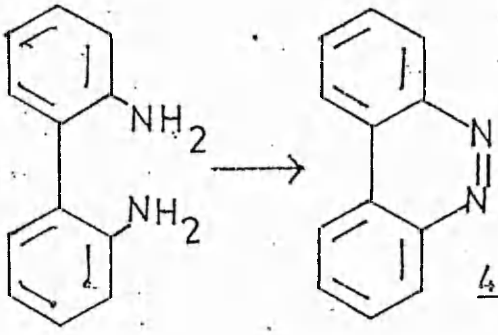
The only instances of oxidative coupling of nitrogen functions leading to spirodienones are the formation of spirolactams 216 by oxidation[135,138] of biphenyl-2-carboxamides with persulphate, and production of erysodienone-type compounds 320, 328 and 331 by ferricyanide oxidation of the corresponding amines.[212,218,227].

It was thought possible that the compounds 409 (R = H, alkyl, aryl or acyl) might undergo an intramolecular oxidative cyclisation to afford dienones of the type 444, (for a discussion of the oxidation of the primary amine 410 see Section 3.4). Secondary nitrogen functions were thought to be preferable substrates for oxidation considering the results described above[322] and the wide range of products which have been obtained[306] by manganese dioxide oxidation of primary amines. The compounds chosen were those in which the substituent R in structure 409 was: benzoyl, compound 416; toluene-4-sulphonyl, compound 417; benzyl, compound 418; and 2,4-dinitrophenyl, compound 419. The reagent used was manganese dioxide, which had proved successful in the oxidation of 408, and which had also been the best of a number tested in the oxidation of 410 (see Section 3.4); the conditions were similar to those used with 408.

The oxidation of 418 gave mixtures containing between nine and twelve components, none of which corresponded to starting material; this result is hardly surprising in view of the findings of Henbest and his co-workers[323] that N-alkylanilines are oxidised by manganese dioxide to several products, and also that N-benzylanilines have been reported[324,325] to be specifically converted by the same oxidant to benzylidene anilines.

Oxidation of 416 gave a brick-red amorphous solid which had unresolved infrared and n.m.r. spectra but was homogeneous by t.l.c., with an R_f value similar to that of the starting amide. Mass spectroscopy showed it to contain an appreciable amount of 416 and it is probable that the material was a mixture of 416 and a red oxidation product which had similar chromatographic behaviour. Reduction of the solid by sodium borohydride led to disappearance of the red colour but no product was isolated.

Oxidation of 419 gave a mixture containing one major and one minor product together with unchanged 419. Preparative t.l.c. led to isolation of a small amount of the major component but not in an



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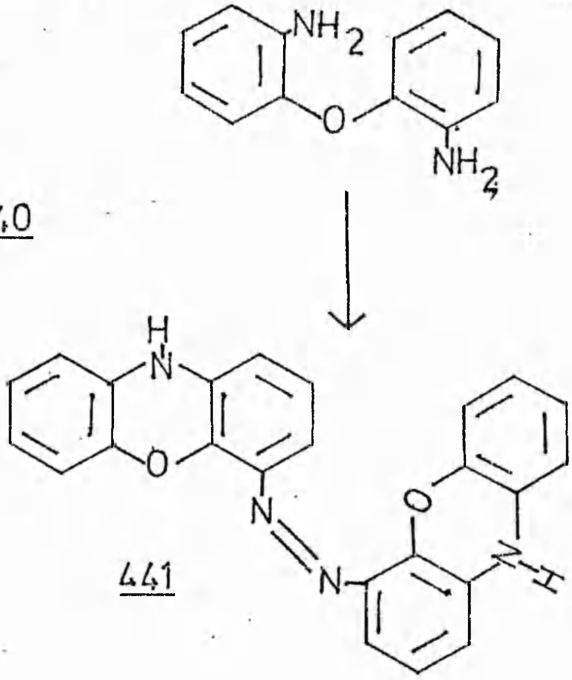

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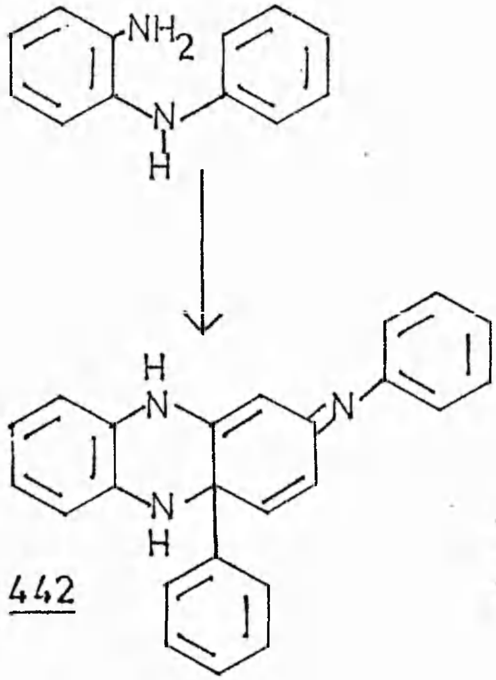

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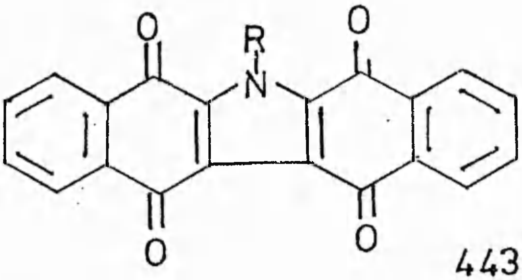

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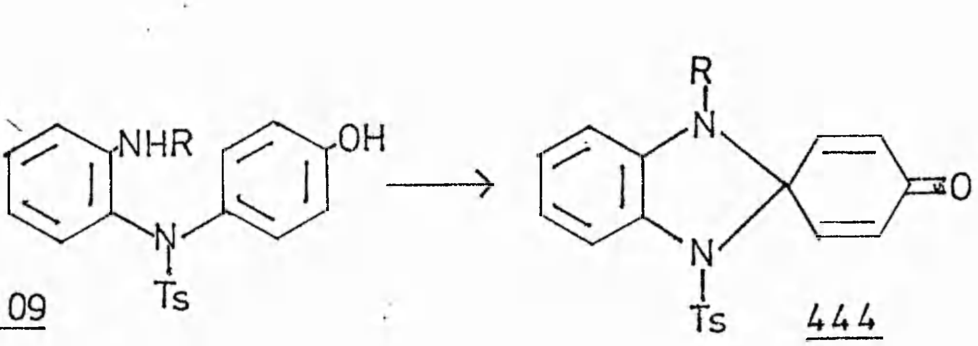


409

444

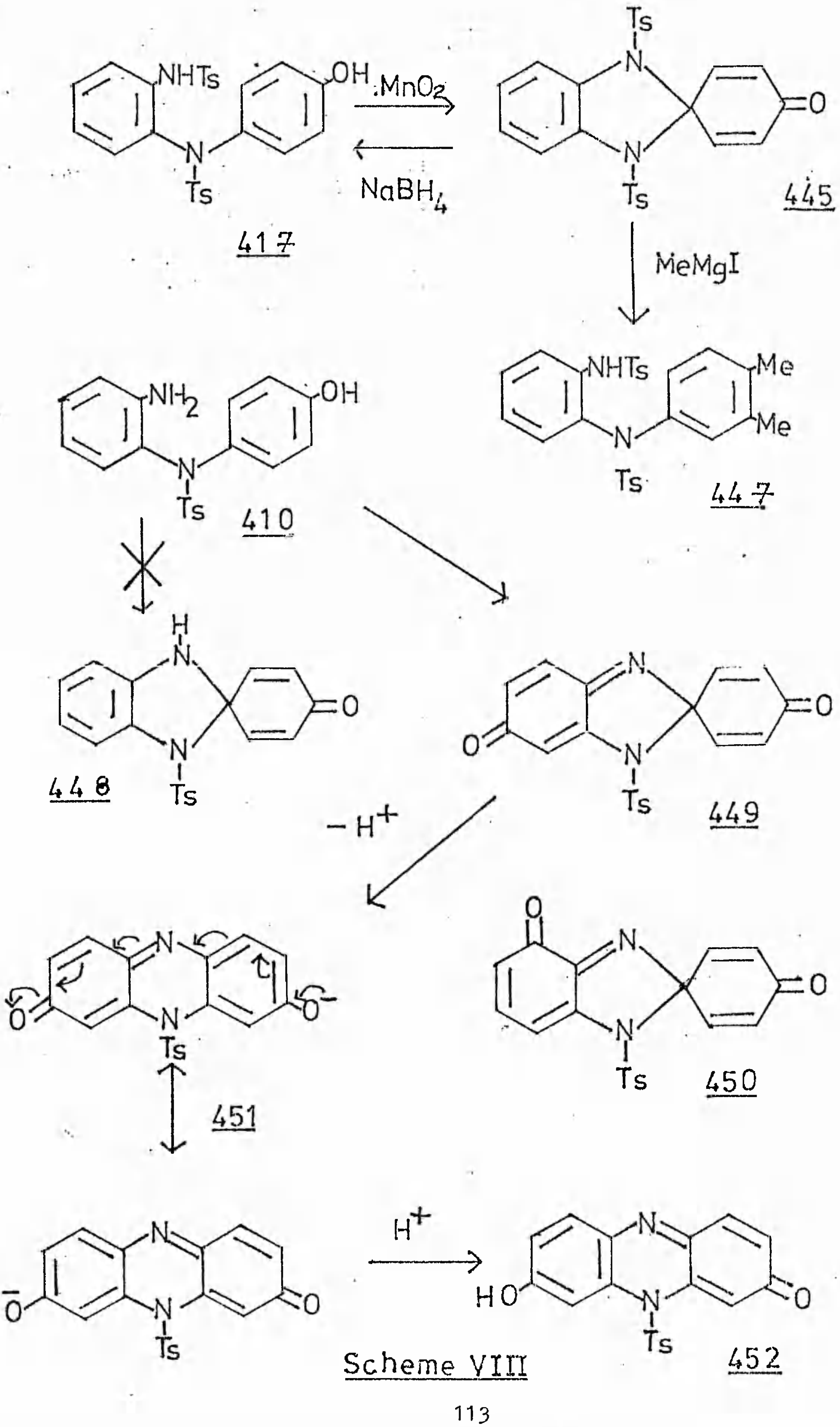
absolutely pure state. Infrared spectroscopy showed the presence of a carbonyl absorption and it is possible that this material contained a spirodienone; lack of time prevented repetition of this oxidation on a sufficiently large scale to enable a reasonable amount of the product to be obtained pure.

Oxidation of 417 led to the formation of N,N′-bis-(toluene-4-sulphonyl)-2,3-dihydro-1H-benz[d]imidazole-2-spiro-1′-cyclohexa-2′,5′-dien-4′-one 445 in 58% yield. This dienone was highly crystalline and its spectral properties were similar to those of 430; it showed infrared absorptions at 1682, 1643 and 1618 cm^{-1}, while the olefinic protons appeared in its n.m.r. spectrum as a quartet between 3.40 and 3.90 τ with $J_{AB} = 10.1$ Hz. Its reactions were also similar to those of 430, and it did not undergo a dienone-phenol rearrangement on treatment with acetic anhydride containing either sulphuric acid or boron trifluoride etherate; in the first case a four component mixture was obtained, while in the second, 445 was recovered unchanged. On reduction with sodium borohydride it afforded the secondary sulphonamide 417 in quantitative yield.

Reaction of 445 with an excess of methylmagnesium iodide gave, after hydrolysis, a mixture of one major and one minor product. High resolution mass spectrometry of the crude mixture showed that the major product was $C_{28}H_{28}N_2O_4S_2$, corresponding to the addition of two methyl groups to 445, while the minor product had the formula $C_{29}H_{30}N_2O_4S_2$ i.e. had been formed by the addition of three methyl groups. The major component was isolated with the aid of preparative t.l.c. and, although its n.m.r. spectrum was ill-defined, it was thought likely that its structure would be analogous to the product from the Grignard reaction of 430. In order to confirm this the secondary sulphonamide 447 was prepared as follows. Reaction of N-3,4-dimethylphenyltoluene-4-sulphonamide with 1-bromo-2-nitrobenzene in the presence of copper afforded N-3,4-dimethylphenyl-N-2-nitrophenyltoluene-4-sulphonamide 397 in 61% yield; subsequent catalytic hydrogenation over palladium gave a 79% yield of N-2-aminophenyl-N-3,4-dimethylphenyltoluene-4-sulphonamide 446 which was quantitatively converted to N-3,4-dimethylphenyl-N-2-(toluene-4-sulphonamido)-phenyltoluene-4-sulphonamide 447. This compound proved to be identical by t.l.c. in two systems, infrared spectrum and mixed m.p. with the major product of the Grignard reaction. The structure of the minor component which had undergone addition of three methyl groups is unknown.

3.4 Oxidation of the Primary Amine 410

As this compound was available in large quantities, its behaviour on oxidation was investigated. The first attempts using the same method that was used for the oxidation of 408, gave mixtures which t.l.c. showed to contain a bright yellow compound in addition to several red and brown components. Preparative t.l.c. led to the isolation of this yellow substance in small amounts. Further oxidations on a larger scale using the same type of manganese dioxide produced little or none of this yellow product and the effect of other oxidants was investigated. DDQ in boiling benzene[116] gave a mixture containing none of the yellow compound, and similar results were obtained using lead dioxide[24] or silver oxide[326] in



Scheme VIII

the same solvent. Manganese (III) acetate in refluxing acetic acid[327,328] produced a mixture containing two components neither of which was either starting material or the yellow compound, while the use of manganese tris-(acetylacetonate) in boiling acetonitrile[329] afforded small amounts of the yellow product together with larger quantities of unchanged 410.

Initially the yellow compound was thought to be the spiro-dienone 448, but the absence of an N-H absorption in the infrared, and its failure to form an N-acetyl derivative on treatment with acetyl chloride in pyridine, together with inconsistencies in its n.m.r. spectrum made this unlikely. Its infrared spectrum showed absorptions at 1678, 1630 and 1608 cm^{-1} while the vinylic region of the n.m.r. spectrum of a carefully purified sample showed a doublet at 3.18 τ, a singlet at 3.35 τ, a doublet at 3.53 τ and singlets at 3.63 and 3.80 τ; the total integral showing 7 olefinic protons and 4 aromatic protons. The elemental analyses of C, H and N were lower than those calculated for the dienone 448. High resolution mass spectrometry gave a m/e value of 366.0701 for the molecular ion which compared well with the value, 366.0675 calculated for $C_{19}H_{14}N_2O_4S$. As can be seen from this formula, an extra oxygen atom has been introduced during the oxidation. The only structures which reconcile these facts are 449 and 450, as structures incorporating N-oxide functions do not account for the seven olefinic protons seen in the n.m.r. Of these structures, the former is the more likely as the α-proton in a cyclohexa-2,4-dienone (c.f. 450) resonates at a value[330] in excess of 4.0 τ, while the highest resonance in the present case is 3.8 τ. In addition the formation of 449 can be rationalised by considering the known[331] formation of 1,4-benzoquinone by manganese dioxide oxidation of aniline; it is possible that the initially formed spirodienone 448, which is formally a substituted aniline, is further oxidised to produce the quinone imine 449.

In an attempt to reduce compound 449 it was treated with sodium borohydride. An intense dark blue colour immediately developed on addition of the reagent and faded after one or two hours. This was a puzzling observation, as borohydride usually reduces unsaturated chromophores, until it was realised that the same intense colour could be produced merely by the addition of dilute sodium hydroxide, and that it was the basic property of the borohydride that produced the effect. The blue colour produced by base was stable for at least 48 hours, and on acidification of the basic solution and extraction into chloroform a red substance was obtained which had an R_f value different from that of the starting yellow compound. This red substance was an unstable gum which tended to decompose into an insoluble brown material on storage, but if freshly prepared it acted as an acid-base indicator, being dark blue in alkaline solution, and a red suspension in acidic media. Infrared spectroscopy of the red gum suggested that a phenol group was present, while the n.m.r. spectrum, although not well resolved, showed a diminution in the number of olefinic protons together with an increase in the number of aromatic protons. One explanation of the above facts is shown in Scheme VIII. If the dienone rearranges under the influence of base to the phenazine derivative 452, the corresponding anion, 451, would be extensively resonance stabilised and might have the low energy electronic transitions necessary to account for its intense visible absorption. The delocalisation of the anion 451 could explain its stability in basic solution, whereas the parent phenol 452, a quinone imine decomposed rapidly in air. The mechanism whereby 449 could rearrange in base to the anion 451 is, however obscure. There is scope for further work to confirm or disprove the latter hypothesis and also to explore more fully the reactions of the interesting structure 449.

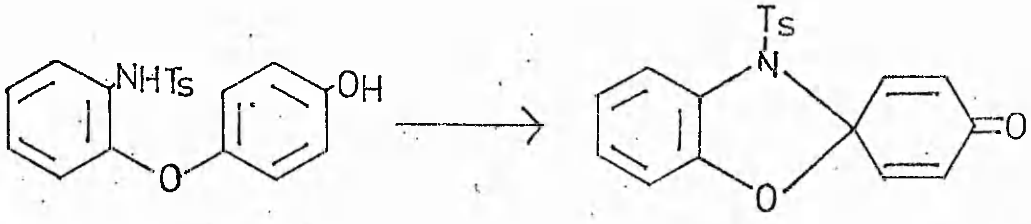
3.5 Oxidation of Compounds 422, 426 and 429

On consideration of the results obtained in Section 3.3, which showed that the nitrogen atom of a secondary sulphonamide underwent ready oxidative cyclisation to a spirodienone, it was realised that there was an alternative route to the preparation of the dienone 430. 4-Hydroxy-2′-(toluene-4-sulphonamido)-diphenyl ether 426, when heated in benzene with active manganese dioxide afforded a 49% yield of the spirodienone 430 which was identical by t.l.c. in two systems, infrared spectroscopy and mixed m.p., with the product of oxidation of 408.

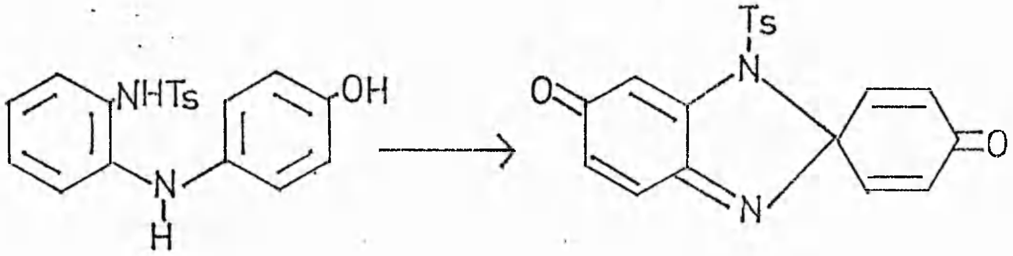
It was also of interest to oxidise 4-hydroxy-2′-(toluene-4-sulphonamido)-diphenylamine 422 to determine whether the dienone 449 would be formed. In fact oxidation of 422 gave a mixture of five components, the major one of which had the same R_f as the yellow compound 449. Preparative t.l.c. led to its isolation in

38% yield and the material was identical, by t.l.c. in two solvents, infrared spectrum and mixed m.p. with that described in Section 3.4. It also behaved in a similar fashion when treated with aqueous alkali, giving an intense blue solution, which, on acidification and extraction, afforded a red substance which had the same R_f value as that of the similar material previously described. This approach to compound 449, although involving more steps, gives much higher yields of product in the crucial oxidation step and would seem to be preferable.

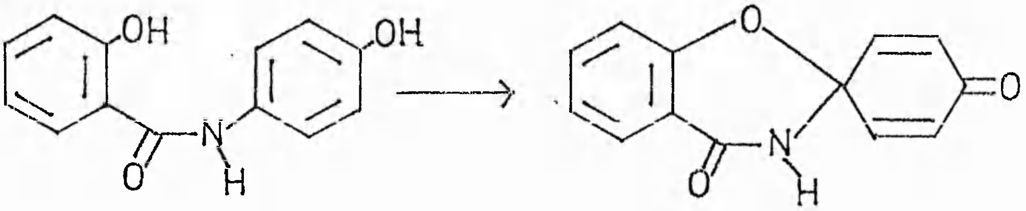
The oxidation of 2,4'-dihydroxybenzanilide 429 was also briefly examined. The product was almost homogeneous but amounted to only 20% of the weight of starting material; this is unusual amongst those compounds oxidised in that the recovery of material is usually between 60 and 80%. Preparative t.l.c. afforded a small amount of a solid which, although homogeneous by t.l.c. would not crystallise. Its infrared spectrum, with absorptions at 1680, 1647 and 1614 cm^{-1}, suggested it might be the spirodienone 453. Lack of time prevented this compound being obtained in sufficient quantity to enable it to be fully characterised.



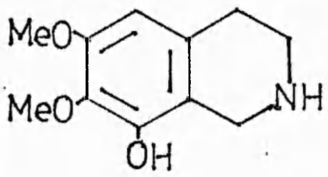
426 430



422 449



429 453



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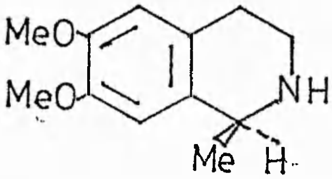

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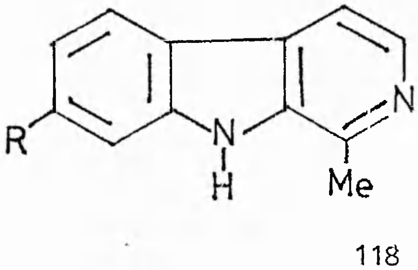

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CHAPTER FOUR

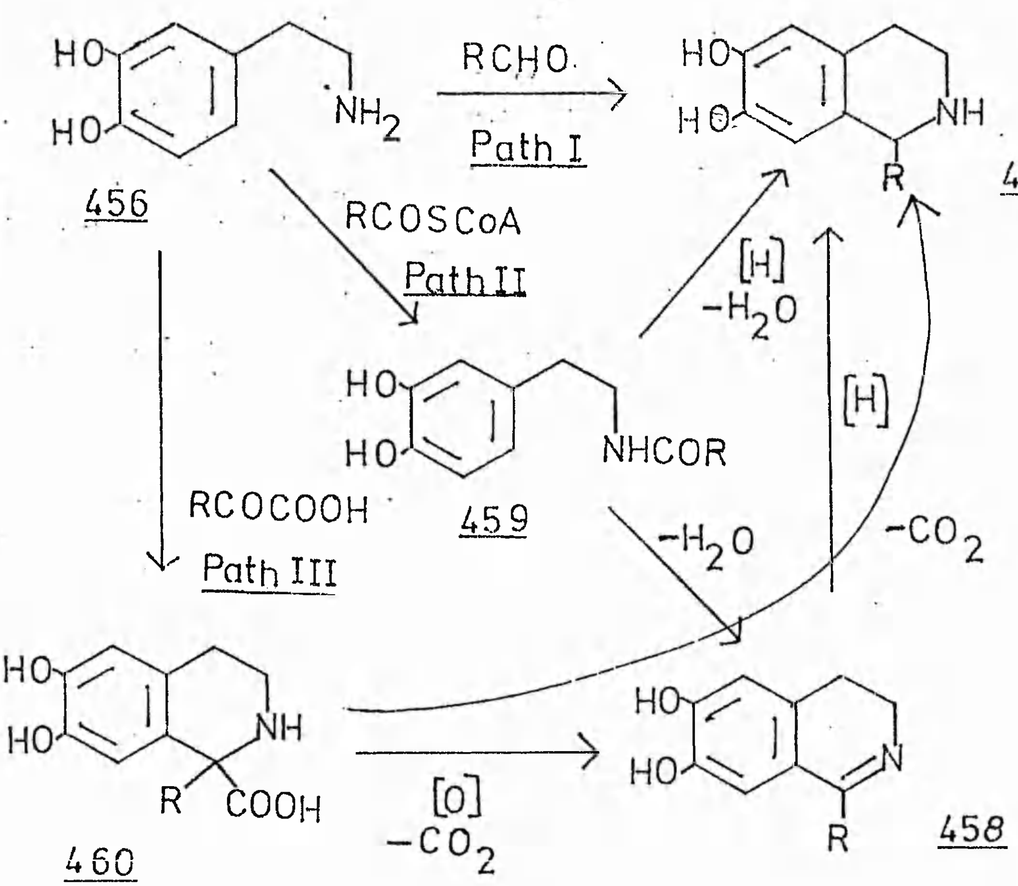
THE ENZYME-CATALYSED DECARBOXYLATION OF ISOQUINALDIC ACIDS

4.1 The Role of Isoquinoline-1-carboxylic acids in The Biogenesis of Isoquinoline Alkaloids

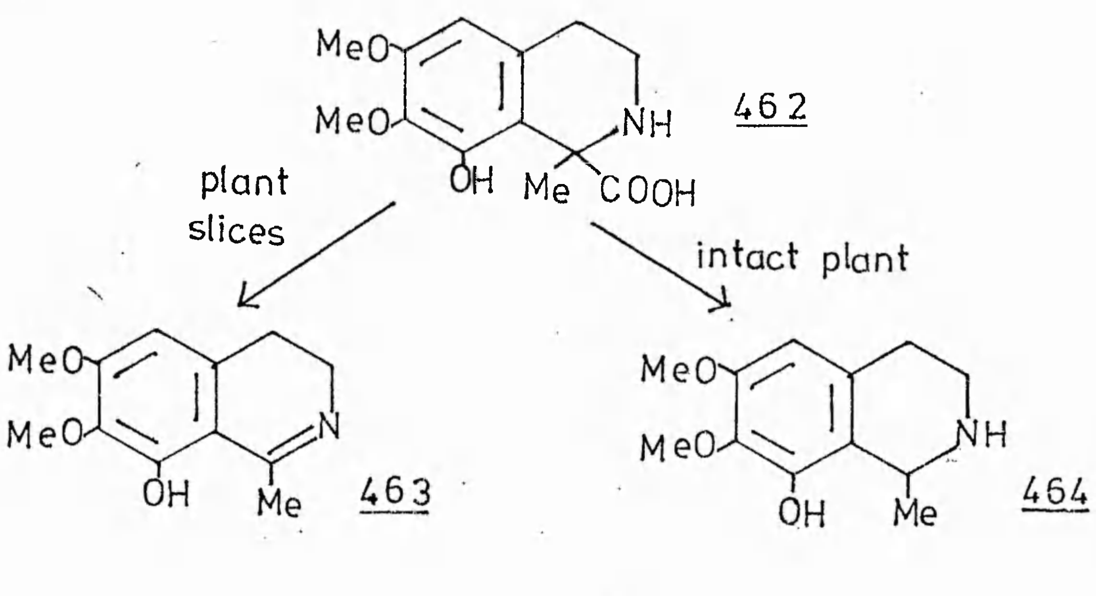
It is generally accepted[332,333] that isoquinolines are formed in vivo by condensation of β-phenethylamines, (derived from tyrosine or dopa), with appropriate carbonyl compounds. The nature of the carbonyl compounds involved has been the subject of much dispute, and the three main mechanisms which have been proposed for the incorporation of the C-1 unit and its attached groups into the isoquinoline molecule are outlined in Scheme IX.

Following the discovery by Pictet and Spengler[334] that phenethylamines react with aldehydes under "physiological" conditions to give tetrahydroisoquinolines, it has been suggested[334,335] that the source of the C-1 moiety is a simple aldehyde, e.g. formaldehyde[334] for alkaloids of the anhalamine 454 type, acetaldehyde[335] for those of the salsolidine 455 family, and substituted phenylacetaldehydes[336] for the 1-benzylisoquinoline alkaloids. This proposal is represented by the conversion 456 → 457 (Path 1) in Scheme IX. Experimental evidence for Path 1 is slight as aldehydes are not generally available in metabolic pathways. Tyrosine and dopa are precursors for both parts of the 1-benzylisoquinoline molecule in several alkaloids,[337] but dopamine is only incorporated into the top half;[338] if a phenylacetaldehyde were to be formed by deamination of dopamine, one would expect the latter to be a precursor for both parts.

A second mechanism, Path II, involves the initial acylation[339]


HO
HO
NH2
456
RCHO
Path I
HO
HO
NH
R
457
RCOSCoA
Path II
[H]
-H2O
HO
HO
NHCOR
459
[H]
RCOCOOH
Path III
-H2O
-CO2
HO
HO
NH
R
COOH
460
[O]
-CO2
HO
HO
N
R
458

Scheme IX

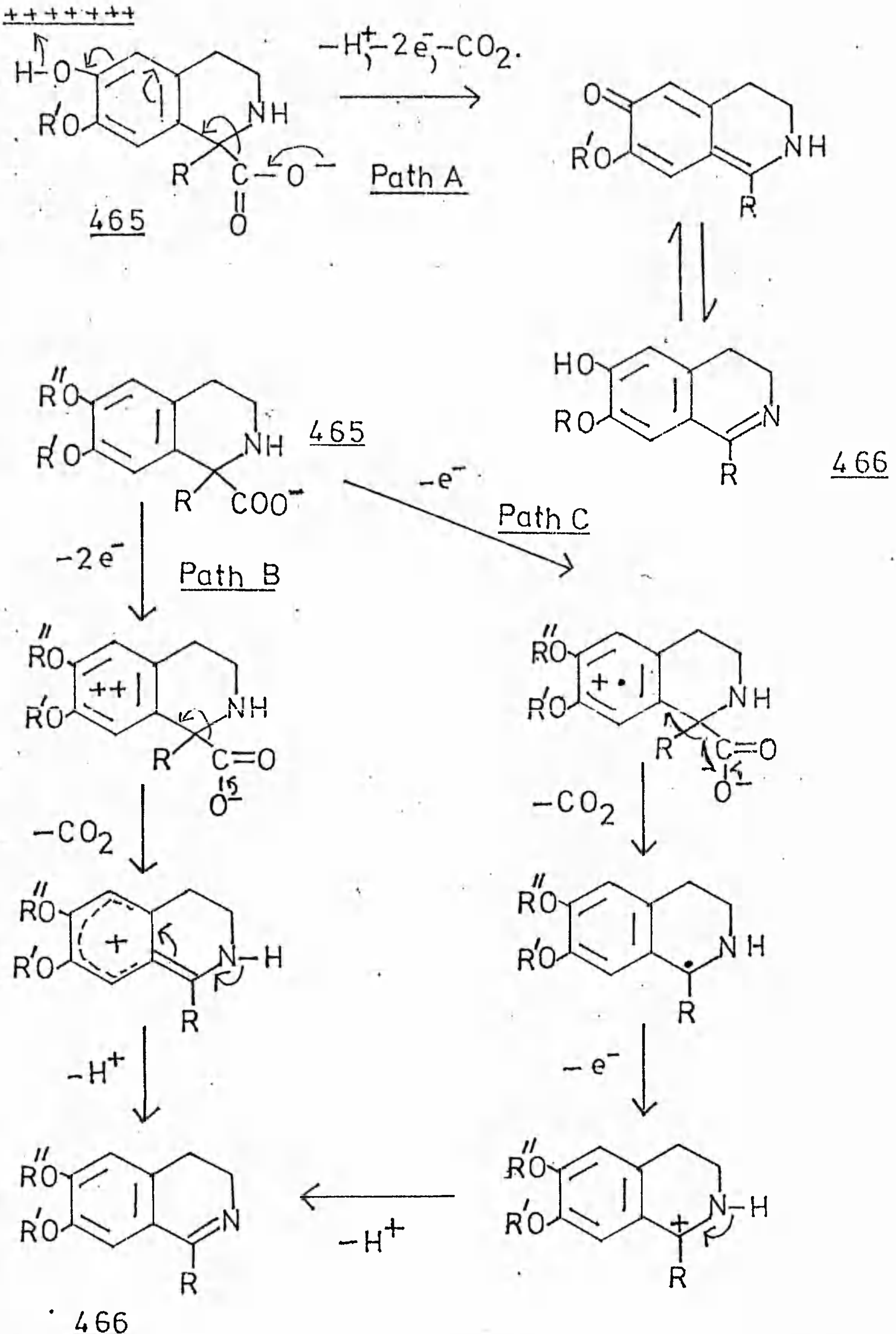

MeO
MeO
NH
OH
Me
COOH
462
plant slices
intact plant
MeO
MeO
N
OH
Me
463
MeO
MeO
NH
OH
Me
464

of the phenethylamine (e.g. by acetyl coenzyme A in the case of the salsolidine alkaloids), followed by cyclisation of the resulting amide 459 to give either 457 or 458. The incorporation[340] of N-acetyltryptamine into harman 461 (R = H) has been adduced as evidence for this mechanism, but other workers[341] have found that an N-acetylphenethylamine was first deacetylated before being incorporated into a peyote alkaloid. Thus the evidence for this pathway is at the best inconclusive.

The third path outlines the proposal by Hahn *et al*[342] that the carbonyl compound is an α-ketoacid, which condenses with the phenethylamine in a Pictet-Spengler-type reaction to afford an isoquinoline-1-carboxylic acid 460; subsequent decarboxylation of this acid produces 457 or 458. This mechanism was at first largely ignored following the failure of Whalley and Govindachari[343] to decarboxylate isoquinaldic acids under mild conditions *in vitro*, but has recently found considerable experimental support. Pyruvate is an efficient precursor of various peyote alkaloids,[339] and of the indole alkaloid, harmine 461 (R = OMe), while isoquinoline-1-carboxylic acids have been isolated from peyote cacti.[341] 3,4-Dihydroxyphenylpyruvic acid has been shown to be a precursor of norlaudansoline[345] and reticuline,[346] while isoquinoline-1-carboxylic acids have been found to be incorporated into several isoquinoline alkaloids.[341,345,347] Incubation of peoruvic acid 462 with fresh cactus slices led to the isolation of the 3,4-dihydroisoquinoline 463, while feeding of 462 to the intact plant gave the tetrahydroisoquinoline alkaloid 464.[341] This result led to the proposal[341] of an oxidative decarboxylation step in isoquinoline biogenesis to be followed by a reduction (e.g. by NADPH).

A recent paper[348] gives details of the decarboxylation of a series of 1-benzyltetrahydroisoquinoline-1-carboxylic acids 465 to produce 3,4-dihydroisoquinolines 466 in high yield, by anodic oxidation in either methanol or aqueous methanolic sodium bicarbonate solution. A free phenol group in either the 6- or 7-position of the isoquinoline nucleus was found to be necessary for smooth decarboxylation at low potential. Possible mechanisms are shown in Scheme X. Path A is a concerted two-electron oxidation of a phenol, but only applies when the hydroxyl group is in the 6-position. Path B is a concerted two-electron oxidation in which the electrons are taken from the ring at the same time as carbon dioxide is lost, while path C is a conventional ECE process similar to one proposed[349] for the "pseudo-Kolbe" reaction. Since there is little difference between the 6- and 7-hydroxyacids in the ease of decarboxylation, path A is insufficient to account for the reaction and another mechanism (path B or C) must operate. Further work from the same laboratory[350] has shown that similar isoquinoline-1-carboxylic acids are also decarboxylated under the influence of aerial oxygen and alkali but the reaction is slower than the electrochemical one and gives more than one product.

In view of the low anodic potential required to decarboxylate 6- or 7-hydroxyisoquinaldic acids, it seemed likely that such compounds would undergo a similar reaction on treatment with enzymes known to oxidise phenols. If this were so, it would be evidence in favour of the proposal[351] that certain electrochemical



Scheme X

oxidations give similar results to those occurring in vivo. The enzymes chosen for this work were fungal laccase and horseradish peroxidase.

4.2 Occurrence and Properties of Laccase and Peroxidase

4.2.1 Laccase

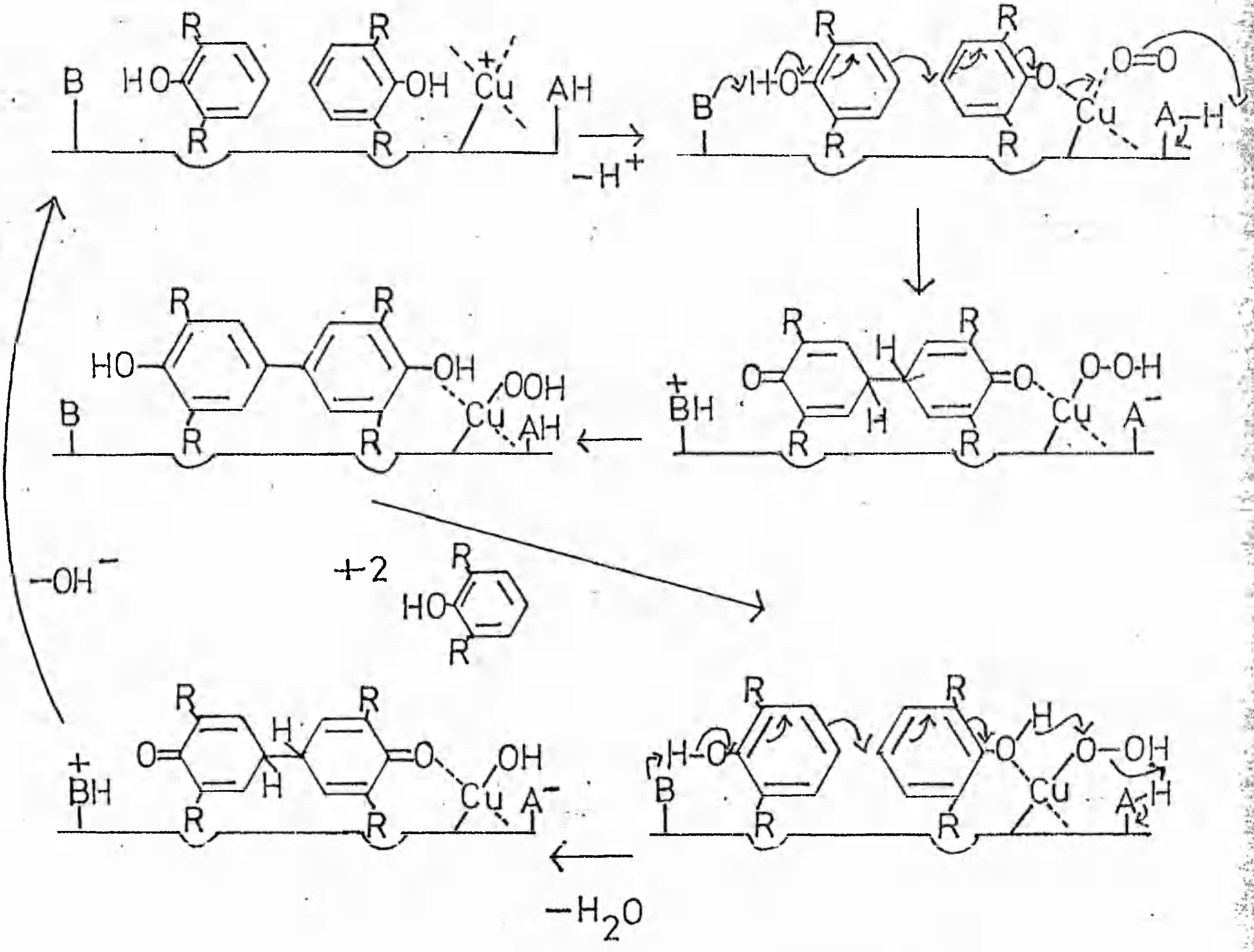
Laccase is a member of the group of enzymes known as the blue oxidases. These are copper-containing glycoproteins which owe their blue colour to the visible absorbance of a Cu(II) ion, and comprise ascorbate oxidase (EC 1.10.3.3.), ceruloplasmin or ferroxidase (EC 1.16.3.1.), tree laccase and fungal laccase. Confusion exists between laccase and monophenol monooxygenase (or tyrosinase, (EC 1.14.18.1.) which, in addition to introducing oxygen into a phenol, can also catalyse reactions similar to those catalysed by laccase. In the Enzyme Commission recommendations (1965) laccase was named p-diphenol:oxygen oxidoreductase (EC 1.10.3.2.) but this listing has been deleted in the latest (1973) recommendations.[353]

Tree laccase is obtained from the Japanese lac tree (Rhus vernicifera)[354] and occurs together with another blue copper-protein, stellamycin,[355] while fungal laccase occurs in several fungi,mainly Basidiomycetes[356] and Ascomycetes.[357] Some fungal laccases are produced extracellularly and a proposal has been made that their function is to break down lignin in the wood on which the fungi grow, to form metabolically useful products. Most work has been done with the enzymes from two species, Polyporus versicolor[358] and Podospora anserina,[359] although others have occasionally been used.[360-4] The enzyme from Polyporus versicolor is secreted into the medium when grown in liquid culture, and high yields can be obtained when an inducer indole-3-acetic acid[364] or 2,5-dimethylaniline[365] is added to the medium.

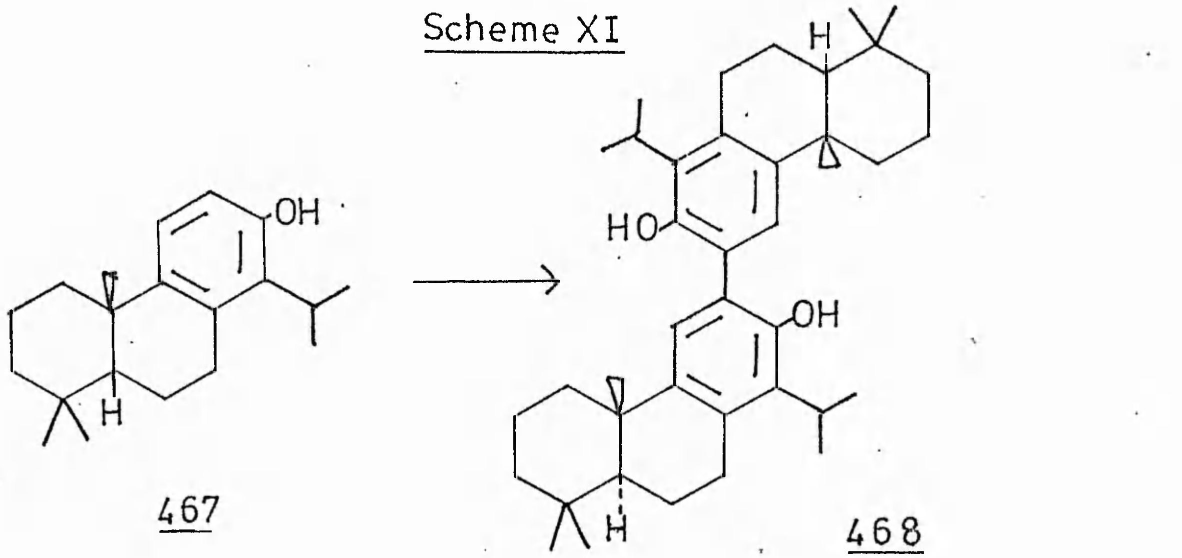
The early literature contains contradictory reports on the substrate specificity of laccase and much work was done to determine whether o-, m-, and p-diphenols and o-, m-, and p-substituted monophenols were or were not oxidised.[360,361,363,366-8]

More recent work has concentrated on the mechanism of action of the enzyme and especially on the type and role of the copper atoms. Fungal laccase contains four copper atoms of which two give EPR signals typical of Cu(II) ions.[369] One of these latter atoms is designated Type 1 copper and is responsible for the blue colour with a visible absorbance band about 600 nm.[370] The other is designated Type 2 and its characteristic property is the ease with which it binds to anionic inhibitors.[371] The two EPR-nondetectable copper atoms are designated Type 3 and were originally thought to be Cu(I) ions to account for their lack of paramagnetism;[372] later work,[373] however, suggests that they exist as a coupled pair of Cu(II) atoms capable of functioning[374] as a two-electron acceptor.

Fungal laccase can accept as many electrons as it has copper atoms[375] but it is not yet clear whether in the normal reaction more than one electron can be taken at one time from one substrate molecule. Free radicals have been detected as intermediates in laccase-catalysed oxidations,[376] but there is a possibility that they may arise from side reactions, as has been demonstrated for tyrosinase.[377] Although the balance of evidence favours[352] one-electron oxidations, a mechanism has been proposed[378] (Scheme XI)



Scheme XI



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in which laccase could effect a two-electron oxidation as, for example, the carbon-carbon coupling of phenols, by the intermediacy of a single Cu(II) atom. The copper hydroperoxide formed would react with a further two molecules of phenol and thus the transfer of the four electrons necessary to reduce a molecule of oxygen to two molecules of water would be completed in two two-electron oxidation steps.[379]

Fungal laccase has been used by Bocks and her co-workers[380,381] to effect oxidative coupling reactions in vitro. 2,6-Dimethyl- and 2,6-dimethoxyphenols were converted into the corresponding diphenoquinones in 30 and 90% yields respectively, while pyrogallol gave a quantitative yield of purpurogallin. Enzymic oxidation of griseophenone 280 gave a 37% yield of dehydrogriseofulvin 279, while similar treatment of totarol 467 afforded podototarin 468 in 20% yield.

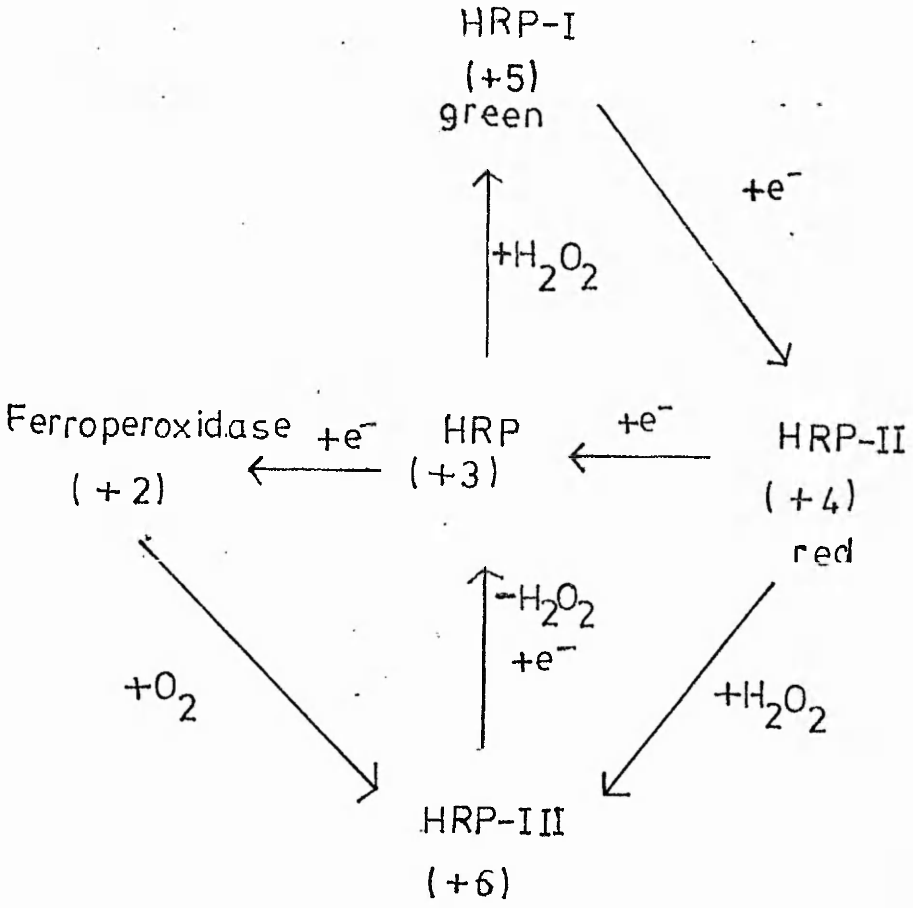
4.2.2 Horseradish Peroxidase

Peroxidases[382] (EC 1.11.1.7, donor: H_2O_2 oxidoreductase) are glycoproteins which have the iron-containing protoporphyrin IX 469 as a prosthetic group. They occur in several animal cells and in virtually all plants, and the enzymes from different sources have molecular weights ranging from 30,000 to 100,000. Most work has been done with the enzyme from the horseradish root; it is 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 studied.

Early work[383,384] showed that the first product(designated HRP-I) was green and that a subsequent one-electron reduction gave a red product (HRP-II), which, after a further one-electron reduction reformed the free enzyme. The compounds I and II were originally thought to be enzyme-substrate complexes, until it was found that HRP-I was not in equilibrium with HRP and, furthermore, HRP-I could be produced by the action of other oxidants (e.g. molybdicyanide).[385] Thus the enzyme reacts with peroxide to form distinct compounds and further work[385] characterised two more of these, HRP-III and ferroperoxidase. The iron atom in HRP is in the Fe(III) oxidation state while in ferroperoxidase it is Fe(II). The interconversion[386] of the compounds is shown in Scheme XII; the numbers in parentheses for compounds I, II and III refer to the "oxidising-equivalents" and not necessarily to the oxidation state of the iron atom. The enzyme can form similar compounds with methyl, ethyl, _n_-propyl and _iso_-propyl hydroperoxides but not with the _t_-butyl compound.[382a]

The iron atom in the porphyrin has six co-ordination sites, four of which are bound to the pyrrole nitrogens and one to the protein itself. One theory[387] of the structure of the HRP compounds suggests that in HRP-I, oxide is co-ordinated in the sixth position with the iron in the Fe(V) state, and when it is reduced to HRP-II the iron is in the ferryl state (FeO^{2+}).[388] An alternative theory[389,390] proposes that the porphyrin ring is oxidised by


HRP-I
(+5)
green
+e⁻
$+H_2O_2$
Ferroperoxidase
(+ 2)
+e⁻
HRP
(+3)
+e⁻
HRP-II
(+4)
red
$-H_2O_2$
+e⁻
$+O_2$
$+H_2O_2$
HRP-III
(+6)

Scheme XII

peroxide in HRP-I and on reduction to HRP-II the iron changes from ferric to ferrous. Comparison of absorption spectra with those of known porphyrins, and magnetic susceptibility studies have not resolved these two theories.[391]

The reaction of HRP compounds with hydrogen donors is universally thought[392] to involve the generation of free radicals as follows:

$$\text{HRP} + \text{H}_2\text{O}_2 \longrightarrow \text{HRP-II}$$

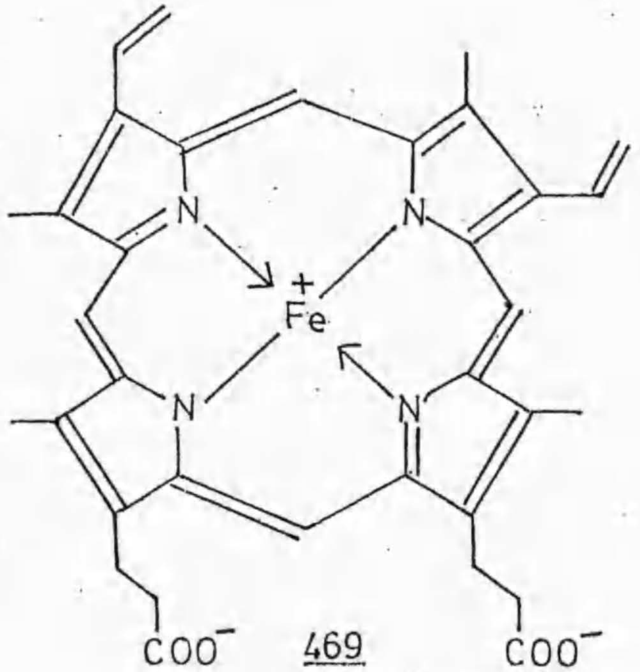
$$\text{HRP-I} + \text{AH}_2 \longrightarrow \text{HRP-II} + \text{AH}\bullet$$

$$\text{HRP-II} + \text{AH}_2 \longrightarrow \text{HRP} + \text{AH}\bullet$$

$$2\text{AH}\bullet \longrightarrow \text{products}$$

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 the saturation kinetics characteristic of enzyme reactions. Recent work[393] has investigated the reaction of compounds I and II with iodide, ferrocyanide, 4-cresol and 4-aminobenzoic acid, with the conclusion that the first two do not form enzyme-substrate complexes with either compound, while 4-cresol forms a complex with HRP-II, but evidence is presented that it may be unproductive. The case of 4-aminobenzoic acid is more complex and enzyme-substrate complexes may be involved. Previously the only instance[394] in which saturation kinetics had been found to apply was the reaction of HRP-II with ferrocyctochrome c.

The range of compounds which can act as hydrogen donors to HRP compounds is wide[395] and includes aromatic amines, phenols, enediols, leuco dyes and amino acids. The biological role of



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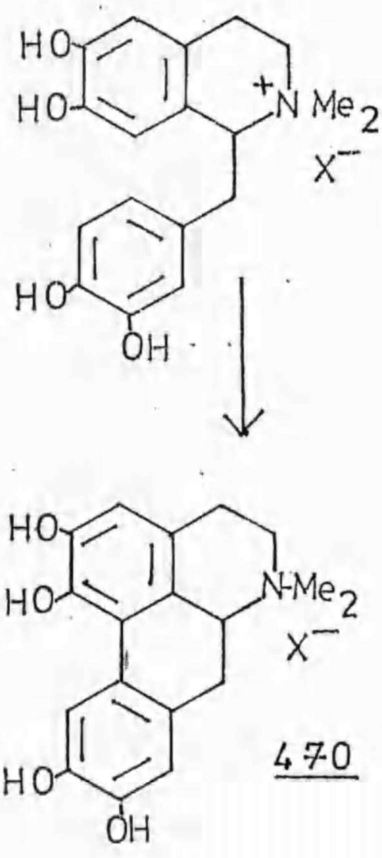

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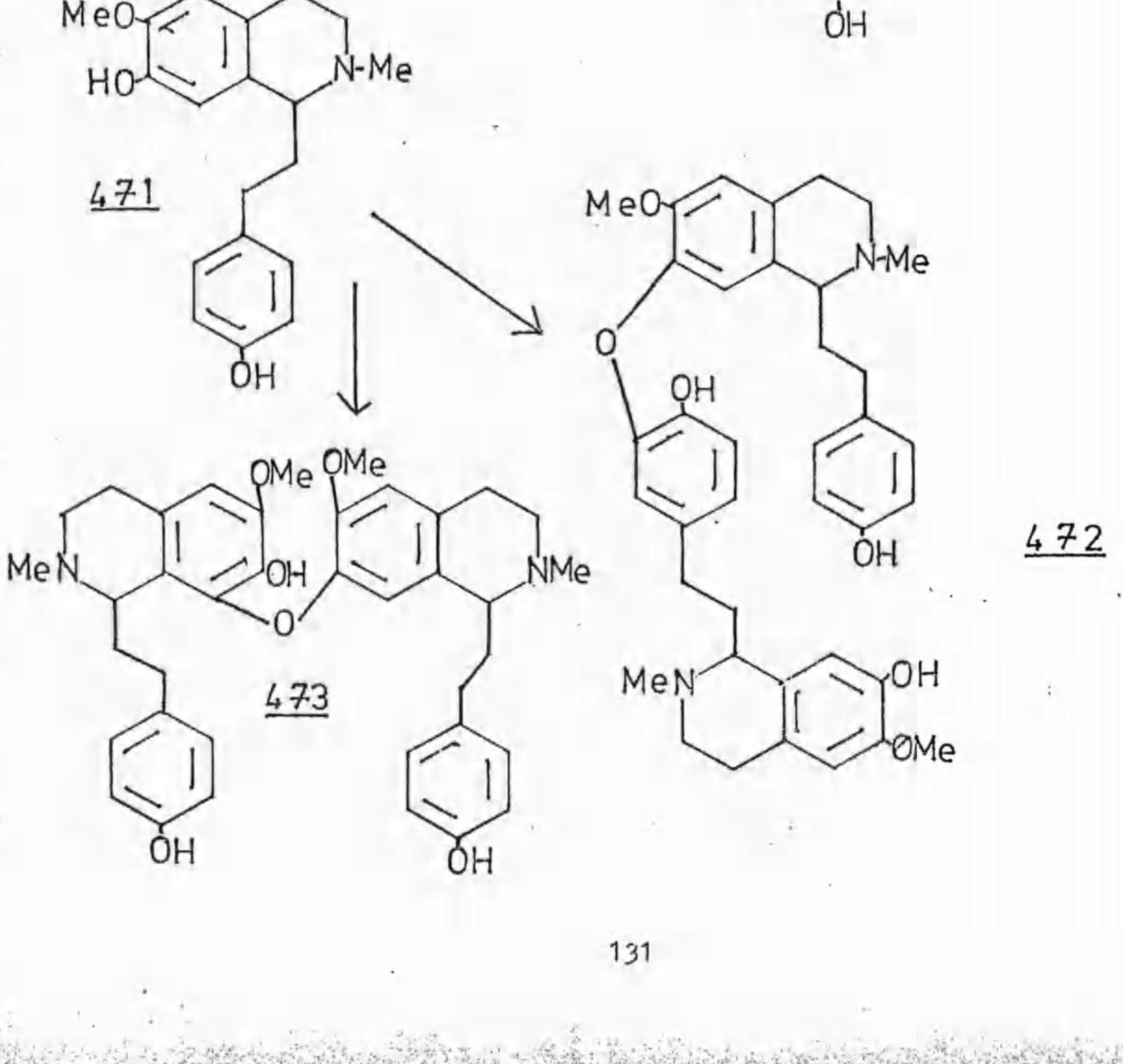

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472

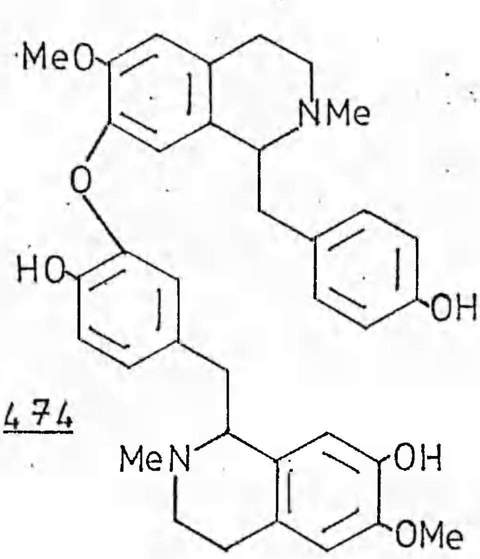
473

peroxidases is unknown.[396] There are proposals that their function is to catalyse phenol oxidative coupling (e.g. in the biosynthesis of lignin[397]) or to remove hydrogen peroxide from the cell;[396] other authors have suggested that peroxidases may be involved in the hydroxylation of aromatic compounds,[398] and the decarboxylation of amino acids.[399]

The synthetic applications of oxidations catalysed by peroxidase are fairly numerous and only those in the isoquinoline alkaloid field will be discussed here. In 1967 the oxidation of laudanosoline methobromide by the enzymes, mushroom tyrosinase, Rhus laccase and horseradish peroxidase was investigated;[400] chromatographic and spectral evidence of the formation of the quaternary aporphine 470 was obtained. Further work was carried out by Kametani and his co-workers who used mixtures of crude plant homogenates and hydrogen peroxide as oxidants. Treatment of the phenethylisoquinoline 471 with homogenised potato peels and peroxide produced[401] the head-to-tail coupled dimer 472 in low yield, while the use of a Wasabia Japonica homogenate afforded[402] a 25% yield of the head-to-head dimer 473. The authors proposed that different enzymes, specific for each coupling, were present in the plants. Oxidation of N-methylcoclaurine with the potato peels system was reported[403] to give very small yields of a head-to-tail dimer 474 and a similar trimer, but only mass spectrometric evidence was obtained for the structures of the products. An attempt[404] to effect coupling of reticuline 308 to salutaridine 307 with a homogenate of Papaver rhoeas (a plant which does not contain morphine alkaloids) and peroxide, led only to hydroxylation of reticuline in the β-position.

The use of pure horseradish peroxidase and peroxide led[405] to cleavage of N-methylcoclaurine and its 7-O-benzyl ether; loss of the 4-hydroxybenzyl grouping gave the corypallines <u>475</u> (R = H or CH_2Ph). Similar treatment of reticuline again formed a cleaved product, thalifoline <u>476</u>. Peroxidase oxidation[406] of armepavine produced O-methylcorypalline <u>475</u> (R = Me), while N-noramepavine gave the dihydroisoquinoline <u>477</u> together with the 1,2-dibenzylisoquinoline <u>478</u> (formed by addition of the 1,4-benzoquinone methide, produced in the oxidative cleavage, to the isoquinoline). The above instances of cleavage when benzylisoquinolines with 4'-hydroxy functions are oxidised enzymatically are in accord with similar observations[407] when anodic oxidation is employed.

Successful oxidative couplings using HRP and peroxide have been achieved; N-methylisosalsoline was converted[406] into a mixture of C-C and C-O-C dimers, <u>479</u> and <u>480</u>, while lophocerine afforded a small yield of the C-O-C dimer <u>481</u>. Similar treatment[406] of N-methylcoclaurine gave a diphenyl ether <u>482</u> together with two dihydroisoquinoline products. An attempt[405] to introduce a second intramolecular ether linkage into the bisbenzylisoquinoline alkaloid, cuspidaline using peroxidase was unsuccessful. Treatment[408] of the aporphine, boldine with HRP and peroxide produced a mixture, from which was isolated 12% of the C-C dimer



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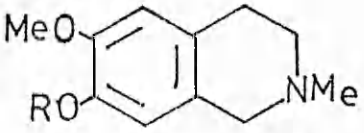

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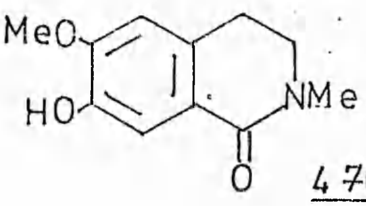

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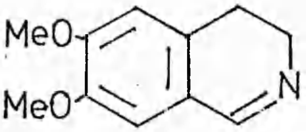

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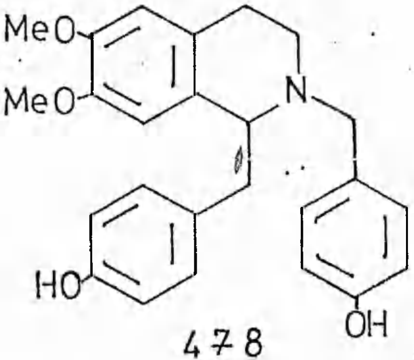

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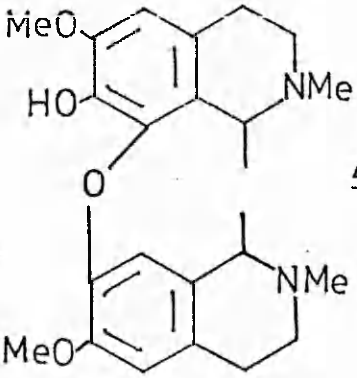

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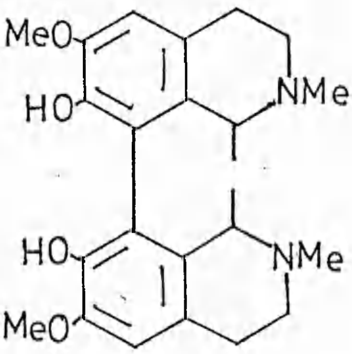

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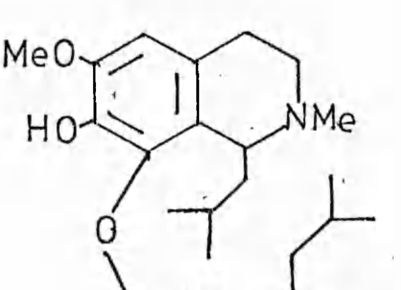


481

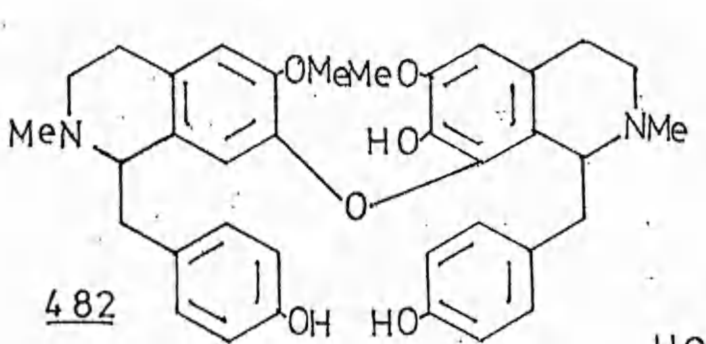
483 and 8% of the C–O–C dimer 484.

Peroxidase oxidation of quaternary bases has given better yields of coupled products; laudanosoline hydrobromide afforded[409] the dibenzopyrrocoline 485 in 81% yield, while laudanosoline methiodide produced[409] the aporphine 470 in 60% yield thus confirming the report of Fromming.[400] The improvement in the reaction when the nitrogen atom is quaternised is in accord with the observations of Franck et al,[410] and is probably due to the nitrogen lone pair of electrons being removed and hence the cleavage of the 1-benzyl group being prevented.

4.3 Preliminary Experiments with Laccase and Synthesis of Substrates

The Basidiomycete, Polyporus versicolor was grown in static cultures of the liquid medium described by Fahreus and Reinhammer.[411] The formation of the extracellular enzyme was induced after 5-7 days growth of the fungus, by addition of 2,5-dimethylaniline. After a further 7 days growth the cultures were harvested and the mycelium-free medium was stored at -20°. The activity of the solutions was assayed using catechol as the substrate,[412] and was generally between 1 and 4 units/cm^3, (for definition of the unit see Experimental). An attempt was made to purify the enzyme by ammonium sulphate precipitation, but this led to the loss of so much activity that it was decided to use the crude culture filtrates.

The crude laccase solution produced dehydrovanillin from vanillin, and 3,3′,5,5′-tetramethoxydiphenoquinone from 2,6-dimethoxyphenol in accord with reports in the literature,[368,380] and did not give a coloured product when mixed with tyrosine,



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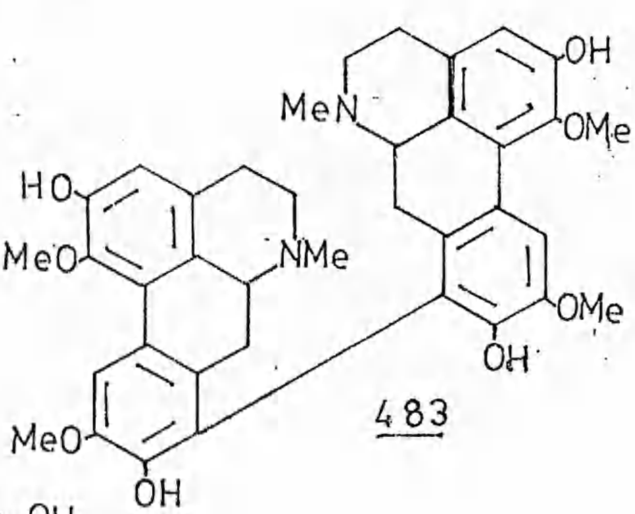

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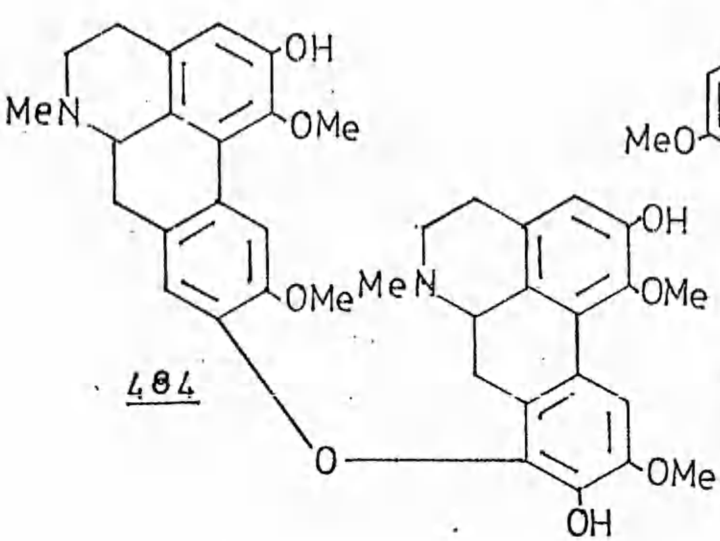

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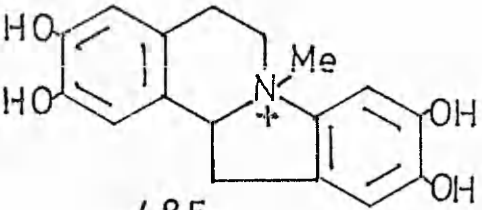

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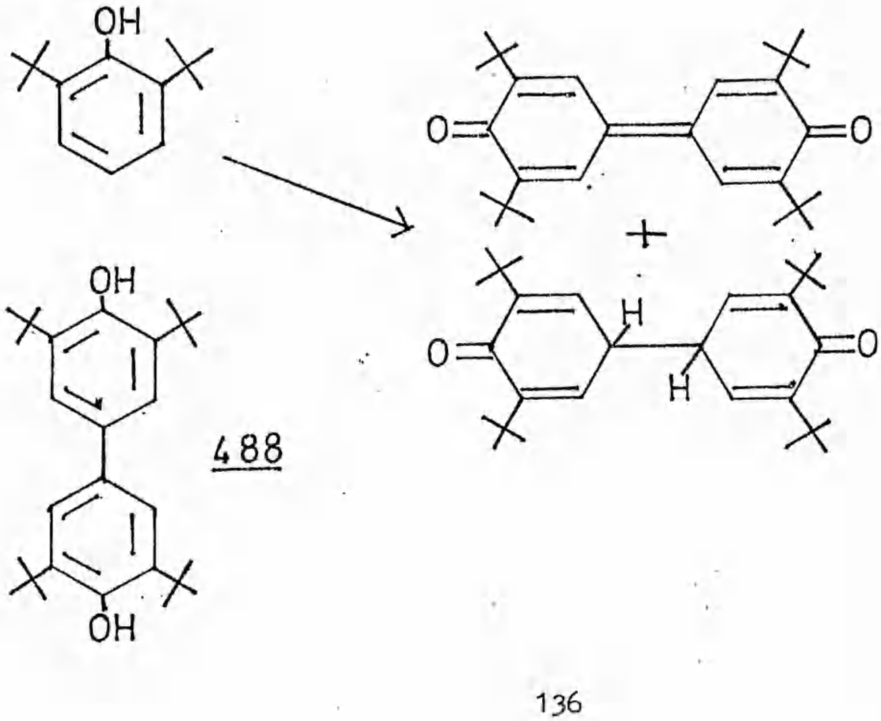

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488

thus confirming[367] the absence of tyrosinase. The effect of the enzyme on 2,6-di-t-butylphenol was investigated; the products were found to be the diphenoquinone 486 and the biscyclohexadienone 487 in almost equal amounts (by t.l.c. comparison of the reaction mixture with samples prepared by literature methods[413,414]). The diphenol 488[415] was found to be absent. Only traces of products were formed when the diphenols 131 and 177 were incubated with enzyme solution, while the dihydroxysulphonamide 408 gave a mixture containing three products in addition to unchanged 408; none of these, however, was the spirodienone 430. The aminohydroxysulphonamide 410 gave a mixture containing five components in addition to starting material; t.l.c. examination showed that none of these was either the yellow compound 449 or likely to be the dienone 448.

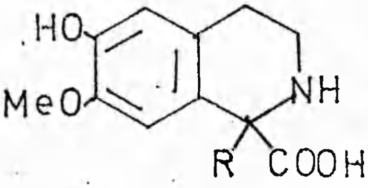
For the decarboxylation studies three types of tetrahydroisoquinoline-1-carboxylic acids were required, viz compounds with 6-hydroxy-7-methoxy-, 7-hydroxy-6-methoxy- and 6,7-dimethoxy-substitution in the isoquinoline nucleus. For each of these groupings it was desired to prepare acids which contained various groups at the 1-position; hydrogen, methyl and benzyl were chosen as substituents. For each carboxylic acid obtained, it was also necessary to synthesise the corresponding 3,4-dihydroisoquinoline, the expected decarboxylation product.

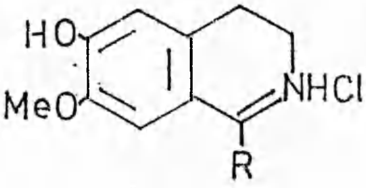
The starting amines for the synthesis of the required acids and isoquinolines, viz 3-benzyloxy-4-methoxy, 4-benzyloxy-3-methoxy- and 3-hydroxy-4-methoxy-β-phenethylamines were prepared according to standard literature methods.[416-8] 6-Hydroxy-7-

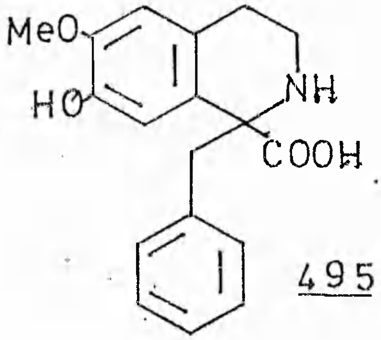
methoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 490 was obtained in 50% yield by Pictet-Spengler condensation between the phenolic β-phenethylamine and pyruvic acid,[419] while the novel acids 489 and 491 were prepared by Mr. E.J. Tinley using analogous condensations with glyoxylic and phenylpyruvic acids respectively. The known[420,421] 3,4-dihydroisoquinolines 492 and 493 were obtained in 46 and 57% yields, by Bischler-Napieralski cyclisation of the N-formyl and N-acetyl derivatives of 3-benzyloxy-4-methoxy-β-phenethylamine, followed by catalytic hydrogenolysis[422] of the resulting 6-O-benzyldihydroisoquinolines. Compounds 494 and 496 were prepared in analogous fashion by Mr. Tinley starting from the appropriate phenethylamines.

The planned route[348] to the 7-hydroxyacids involved the preparation of the Reissert compound from 7-benzyloxy-6-methoxy-3,4-dihydroisoquinoline, followed either by immediate hydrolysis, or by alkylation and then hydrolysis. The hydrolysis, by 85% phosphoric acid, is reported[348] to remove both the O-benzyl and N-benzoyl groups and to convert the nitrile function to a carboxylic acid. Unfortunately, despite numerous attempts by the author and also by Mr. Tinley, the hydrolysis of either the 1-hydrogen, the 1-methyl or the 1-benzyl Reissert compounds could not be effected and a sample of 1-benzyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 495 was obtained from Prof. J.M. Bobbitt.

Two methods were available for the synthesis of the 6,7-dimethoxyacids 497 and 498. In the first, dopamine was condensed

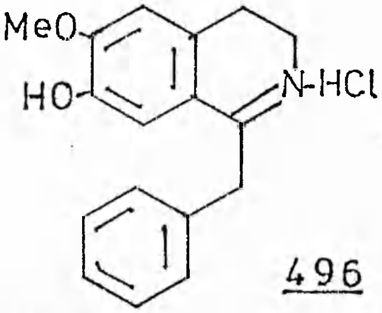






495

489	R=H	492
490	R=Me	493
491	R=CH_2Ph	494



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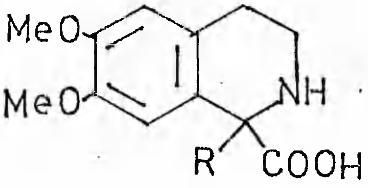
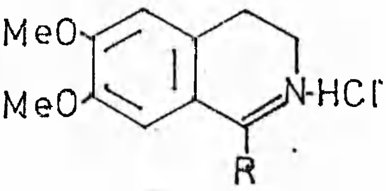
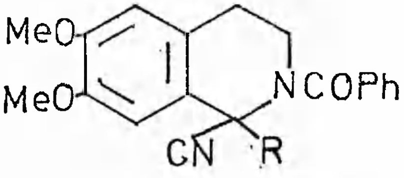
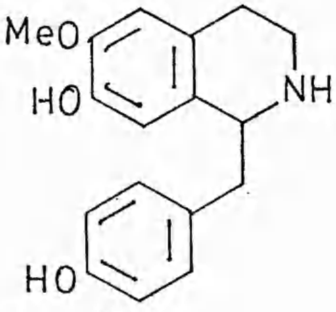




497	R=H	499
498	R=Me	500



501 R=H
502 R=Me



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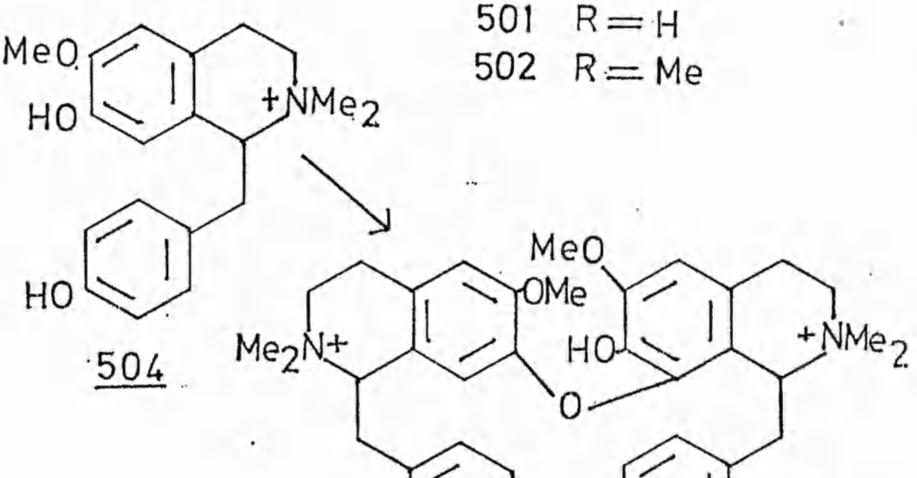

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505

with pyruvic acid under Pictet-Spengler conditions to afford the diphenolic isoquinoline-1-carboxylic acid[423] in good yield; subsequent methylation, however, gave only a poor yield of the dimethoxy methyl ester which could not be hydrolysed cleanly to the dimethoxyacid. The second route proceeded via conversion of 6.7-dimethoxy-3,4-dihydroisoquinoline[424] 499 to the Reissert compound[425] 501 which was hydrolysed to form 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid[426] 497 in 68% yield. Methylation of the Reissert with sodium hydride and iodomethane afforded 2-benzoyl-1-cyano-6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline 502 which was hydrolysed to produce 6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 498 in 24% yield. The dihydroisoquinoline 500[427] was obtained by cyclisation of N-(3,4-dimethoxy-β-phenethyl)-acetamide.

4.4 The Enzyme-catalysed Decarboxylation

As laccase primarily acts on phenols, it was expected that the dimethoxyacids 497 and 498 would not decarboxylate, and they were employed to exclude the presence of any other decarboxylating or hydroxylating enzyme in the crude culture filtrate. Initial experiments investigated the reaction of the enzyme with compounds 490 and 498. The carboxylic acids were soluble in phosphate buffer (pH = 6.0)containing 20% ethanol, and were incubated for 72 hours with addition of enzyme solution after 0, 24 and 48 hours. The reaction mixtures were then made basic by the addition of ammonia solution, and continuously extracted with chloroform. The solution containing the phenolic acid 490 afforded an 18% recovery of a brown gum, the R_f value of which was identical with that of the dihydroisoquinoline 493. Both the gum and the reaction mixture, when viewed under u.v. light, displayed the blue fluorescence characteristic of 3,4-dihydroisoquinolines.

A picrate was formed from the gum and, although it did not melt, a mixture of the picrate and the similar derivative of 493 decomposed at the same temperature as that of the picrate of authentic 493; the two picrates also had the same R_f values. The infrared spectra of both the reaction product and its picrate were identical (although less well resolved) with those of 493 and its picrate. The dimethoxy acid, after incubation with laccase and subsequent basification, did not afford any chloroform soluble material and the reaction mixture did not display the blue fluorescence which was apparent in a solution of 500.

When the dihydroisoquinoline 493 was dissolved in the same buffer system, basified and continuously extracted with chloroform, the recovery was only 30%, which suggested that isolation of the product was not an ideal way to follow the reaction. The possibility that laccase might react further with the dihydroisoquinoline was excluded by incubation of 493 with enzyme solution followed by basification and extraction, when a similar 30% recovery was obtained.

The possibility of following the reaction by u.v. spectrophotometry was next investigated. The dihydroisoquinoline 493 showed absorption maxima at 198, 245, 303 and 369 nm, while the acid 490 had maxima at 209 and 285 nm (both compounds in phosphate buffer solution, pH $\simeq 6.0$). The extinction coefficients of the isoquinoline at 245 and 285 nm were 9850 and 3490 respectively, while those of the acid at the same wavelengths were 1800 and 3230. Using these values the two equations

$$A_{245} = x\,\varepsilon_{245}(\text{acid}) + y\,\varepsilon_{245}(\text{isoq})$$

$$A_{285} = x\,\varepsilon_{285}(\text{acid}) + y\,\varepsilon_{285}(\text{isoq})$$

(where x is the concentration of acid and y is the concentration of isoquinoline) could be solved by measuring the absorbances at the two wavelengths. This method worked fairly well, and the sum of the calculated concentrations of acid and isoquinoline (measured at times during the enzyme reaction) was within 10% of the initial concentration of acid. This showed that the acid was converted to dihydroisoquinoline only and not to additional products, (it is extremely unlikely that any other products should have similar extinction coefficients at the same wavelengths).

The course of the reaction is shown in Fig. I and it can be seen that the reaction is 60% complete after 8 minutes, and slows down markedly thereafter. The acid was stable in buffer solution without added enzyme; after 24 hours u.v. measurements showed the presence of only 5% of dihydroisoquinoline.

The dimethoxyacid <u>498</u> and the dihydroisoquinoline were similarly investigated. The wavelengths used were 243 and 283 nm and the corresponding extinction coefficients were: acid, 3832 and 3364; isoquinoline, 14650 and 4254 respectively. Measurements made during incubation of the acid <u>498</u> with laccase solution showed that after 30 minutes less than 3% of isoquinoline had been formed.

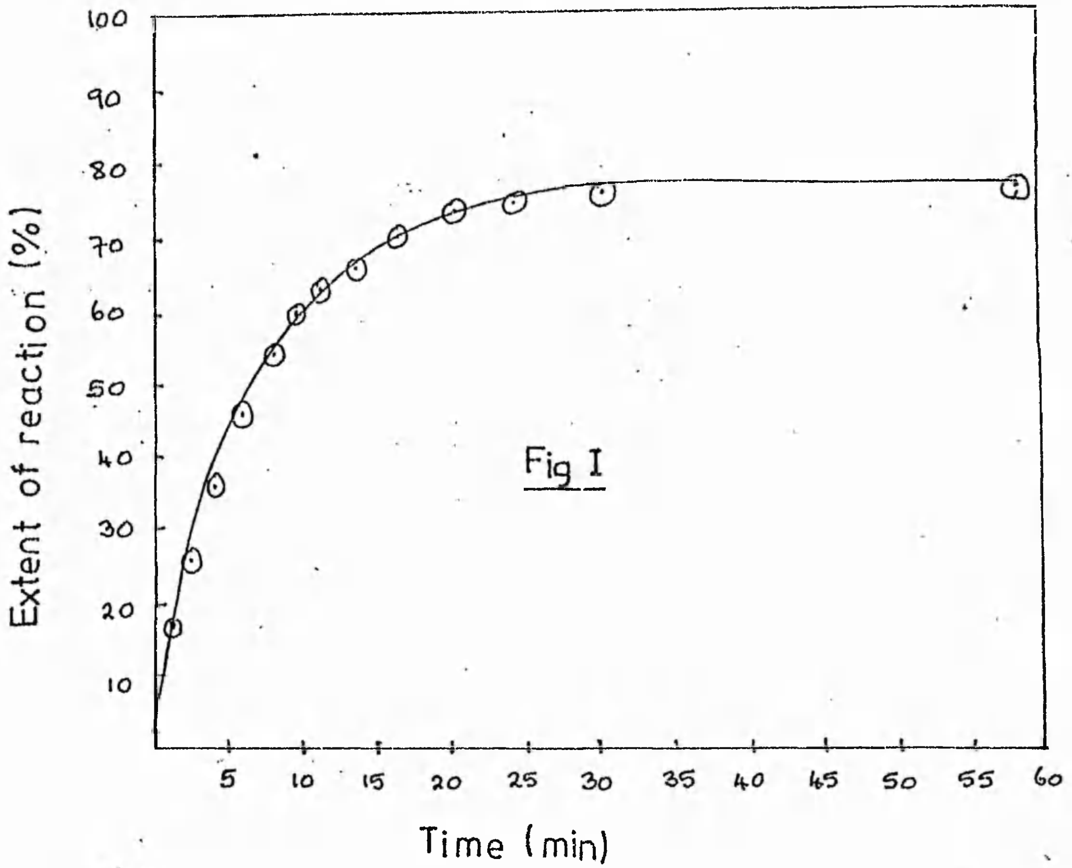

Extent of reaction (%)
100
90
80
70
60
50
40
30
20
10
5
10
15
20
25
30
35
40
45
50
55
60
Time (min)

Fig I

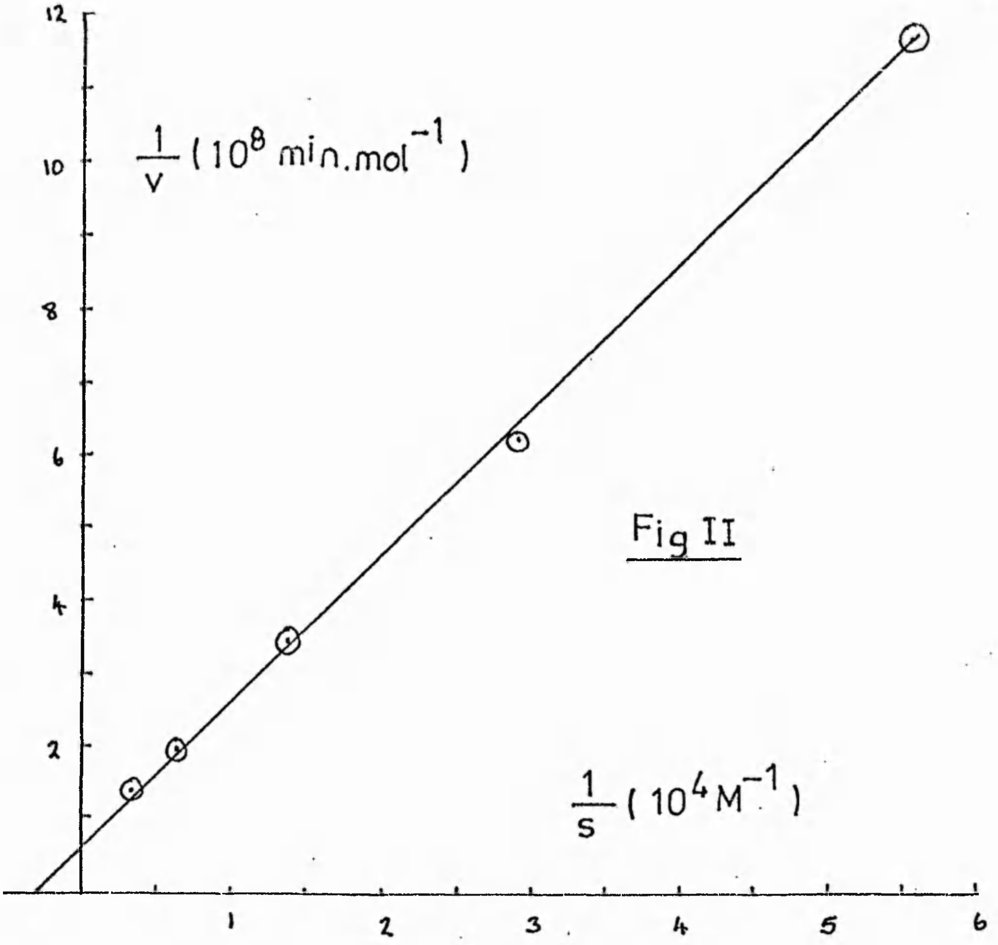

12
10
8
6
4
2
$\frac{1}{v}$ (10^8 min.mol^{-1})
$\frac{1}{s}$ (10^4 M^{-1})
1
2
3
4
5
6

Fig II

Next the rate of decarboxylation of the isoquinoline-1-carboxylic acids was studied. By carrying out the reaction in a silica cell, the increase in absorbance with time at a specific wavelength was recorded on chart paper, and by drawing a tangent to the resulting curve the initial velocity of the reaction could be calculated. For these measurements it was necessary to use a wavelength at which the acid did not absorb, and the wavelengths chosen together with the extinction coefficients of each acid and its corresponding dihydroisoquinoline are given in Table II.

TABLE II

Acid	Isoquinoline	Wavelength nm.	ε_{acid}	ε_{isoq}
489	492	390	30	3360
490	493	368	100	8080
491	494	386	40	9960
495	496	358	80	7540
497	499	356	20	7720
498	500	348	0	6660

The method of carrying out runs is described in the Experimental section, and the initial rates were measured at several different substrate concentrations with constant concentration of enzyme. The results obtained are given in Appendix I. Neither of the dimethoxy acids 497 and 498 showed any increase in absorbance when incubated with enzyme for up to two hours.

In order to test the generality of this enzyme-catalysed oxidative decarboxylation, the reactions with the isoquinoline-1-carboxylic acids were repeated using horseradish peroxidase. This enzyme is commercially available as a crystalline protein and was assayed for activity by the method of Devlin,[428] with the concentrations of the reactants adjusted such that the limiting rate was that of the oxidation of guaiacol. A mixture of peroxidase and hydrogen peroxide was found to catalyse the decarboxylation of the 6-hydroxyacids, which were stable in the presence of either peroxidase or hydrogen peroxide alone. Peroxidase did not affect the 7-hydroxyacid <u>495</u> nor either of the dimethoxyacids <u>497</u> and <u>498</u>. In order to compare the relative ease of decarboxylation of the 6-hydroxyacids by the two enzymes, the initial rates of reaction using peroxidase were determined as described above; the results are given in Appendix I.

In general the initial velocity of an enzyme reaction is given[429a] by the Michaelis-Menten equation

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

where v is the initial velocity, V is the maximum velocity 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 called K_m, the Michaelis constant. The above equation can be written as

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

and it can be seen that a plot of $^1/v$ against $^1/s$ will give a straight line which will intersect the substrate concentration axis at a point giving $-1/K_m$ and the velocity axis at a point giving 1/V. This is the Lineweaver-Burk[430] 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 some doubt of its validity when applied to peroxidase which is reported not to form enzyme-substrate complexes (see Section 4.2.2.) Nevertheless, the runs carried out with the latter enzyme 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 in each case and are shown in Table III.

TABLE III

Acid	K_m (Run 1)	K_m (Run 2)	V (Run 1)	V (Run 2)
	$[10^{-4}\text{M}]$		$[10^{-8}\text{mol.min}^{-1}]$	
a laccase				
489	4.0	3.4	2.0	2.2
490	3.3	3.3	1.43	1.12
491	4.2	4.6	2.94	2.94
495	5.5	8.3	0.83	0.77
b peroxidase				
489	0.42		0.38	
490	1.25	1.0	1.67	1.67
490*	1.19	1.3	0.5	0.85
491	11.2	10.5	11.7	12.5
495	does not react			

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

A representative example of a Lineweaver-Burk plot (that for laccase, run one, with the 6-hydroxyacid 490) is shown in Fig. II. It is apparent that the value of K_m is independent of the enzyme concentration, while the value of V is directly proportional to it; all four pairs of runs using laccase were carried out with identical amounts of the same enzyme solution, and the values of V are directly comparable. The same applies to peroxidase except as mentioned in the footnote to Table III.

Determination of K_m and V enables information to be gained about both parts of an enzyme reaction, the formation of the enzyme-substrate complex, and its subsequent breakdown to give products. The magnitude of K_m is inversely proportional to the affinity of the enzyme for the substrate while the magnitude of V is a measure of the speed at which the complex breaks down.[429b] The data contained in Table III give rise to the following conclusions. Reactions of the 6-hydroxyacids with laccase have similar values of K_m and V, and all would seem to be equally effective as substrates, while the 7-hydroxyacid 495 is both less likely to form a complex with the enzyme and, once it has formed, is slower to break down.

Peroxidase shows much greater specificity, which is in contradiction to what might be expected, considering the reported non-formation of enzyme-substrate complexes, and free radical mechanism. The velocities of reaction of peroxidase with the 1-benzyl, 1-methyl and 1-hydrogen 6-hydroxyacids are in the approximate ratio 100:14:4 and the 7-hydroxyacid does not react at all. The values of K_m for peroxidase are probably not meaningful.

It has, then, been established that phenolic tetrahydroisoquinoline-1-carboxylic acids are oxidatively decarboxylated by enzymes which occur in plant cells, and the results broadly parallel those of anodic decarboxylation, except for the specificity shown by peroxidase. Further work is necessary to elucidate the precise mechanism of the enzymic decarboxylation.

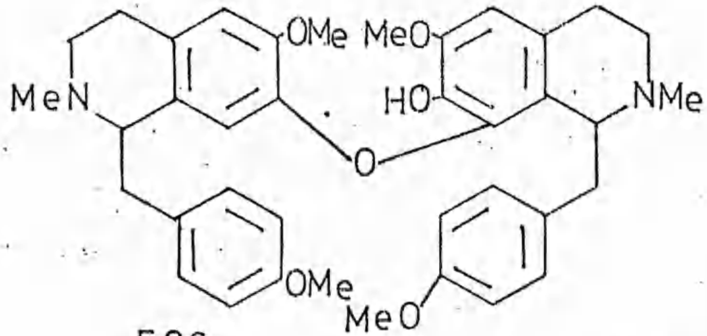
CHAPTER FIVE

THE ANODIC OXIDATION OF A PHENOLIC 1-BENZYLISOQUINOLINE

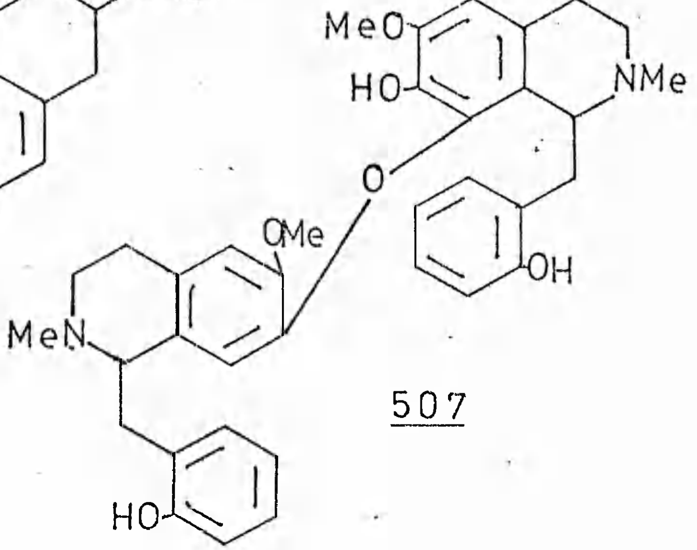
5.1 Intermolecular Coupling of Isoquinolines

It is generally accepted[431] that the biosynthesis of bisbenzylisoquinoline alkaloids proceeds via oxidative coupling of phenolic monomers, mainly derivatives of coclaurine 503. The duplication of these coupling reactions in vitro has, however, not been very successful. The first reported instance was the ferricyanide oxidation[432] of magnocurarine 504 to form the head-to-head dimer 505. Other chemical oxidations have given C-O-C dimers e.g. 506[433] and 507[434] but have not led to any head-to-tail coupled product. Enzymic oxidation has produced both head-to-head and head-to-tail ether linked dimers, (see compounds 472, 473, 482 and 484 in Section 4.2.2). Ferricyanide or silver nitrate oxidation[435] of armepavine methiodide afforded the C-C dimer 508 (R = Me). The anodic oxidation of armepavine and its derivatives has been studied;[436] it was found that the free base and the N-demethyl compound underwent fragmentation, while N-carbethoxy-N-noramepavine produced a mixture containing 45% of the C-C dimer 508 (R = COOEt), and 8% of the tail-to-tail coupled ether 509, which, on reduction, was converted to dauricine.

The oxidative coupling of corypalline and the 1-alkylisoquinolines, N-methylisosalsoline and lophocerine has been more successful. A range of methods have been used including chemical,[437-440] enzymic,[408] photochemical,[441] catalytic[442] and anodic[441-4] oxidations. Two basic types of coupled product are formed, the C-C dimers 510,



506



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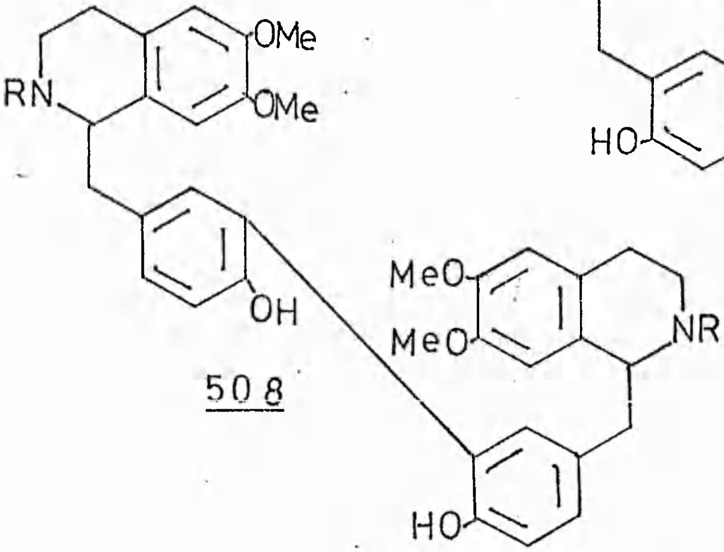

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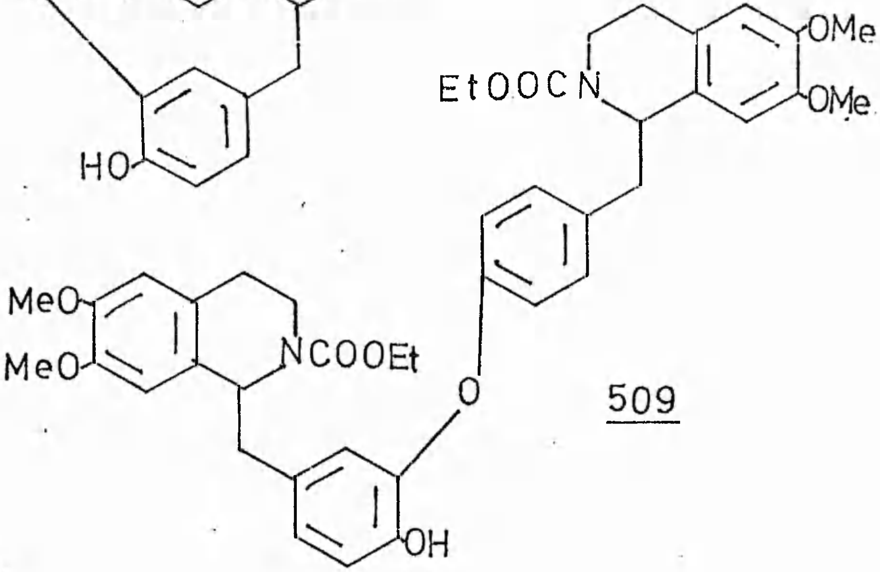
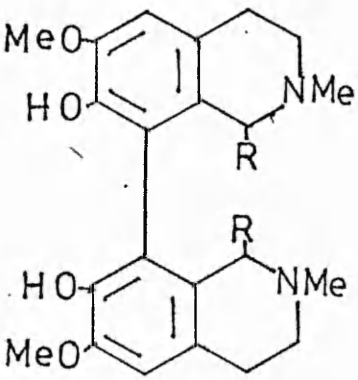


509



510

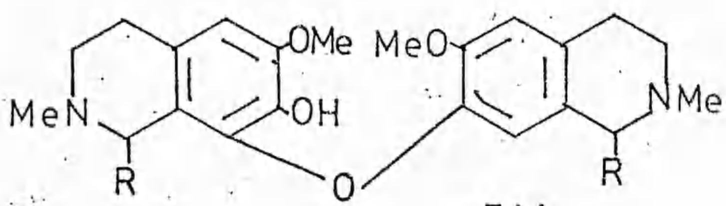
and the C-O-C dimers 511; the type of product formed depends mainly on the R group. When it is hydrogen (corypalline), 510 is the main product; when it is methyl (N-methylisosalsoline), 510 and 511 occur in approximately equal amounts. When R is a more bulky alkyl group such as ethyl[442] or isopropyl (lophocerine), the ether 511 is the predominant product, (sometimes a trimeric ether such as pilocerine is formed[440,442]).

5.2 Bisbenzylisoquinoline Alkaloids with Biaryl Links

Two classes of bisbenzylisoquinoline alkaloids have biaryl links. The first is typified by rodiasine[445] 512 ($R^1 = R^2 = R^3 =$ Me, $R^4 =$ H), first isolated[446] from Ocotea rodiae. Other members of this group are, funiferine[447] 512 ($R^1 = R^2 = R^4 =$ Me, $R^3 =$ H), phlebicine[448] 512 ($R^1 = R^2 =$ Me, $R^3 = R^4 =$ H), and tiliageine[449] 512 ($R^1 = R^2 =$ H, $R^3 = R^4 =$ Me). None of these alkaloids has been synthesised. The second comprises tiliacorine[450] 513 and nortiliacorine A[451] which is an N-demethyltiliacorine. A synthesis of O-methyltiliacorine has been reported.

5.3 Synthesis and Anodic Oxidation of a Phenolic 1-Benzylisoquinoline

On consideration of the work of Bobbitt and Hallcher[436], it was thought possible that anodic oxidation of a 7-O-benzylcoclaurine derivative might afford a C-C dimer 514, which on subsequent methylation and debenzylation would produce a diphenol. A second oxidative coupling step might then introduce a head-to-tail ether link to furnish the rodiasine skeleton. Owing to reports[436] of cleavage in anodic oxidations using the free base, it was thought desirable to remove the nitrogen lone pair of electrons, and in



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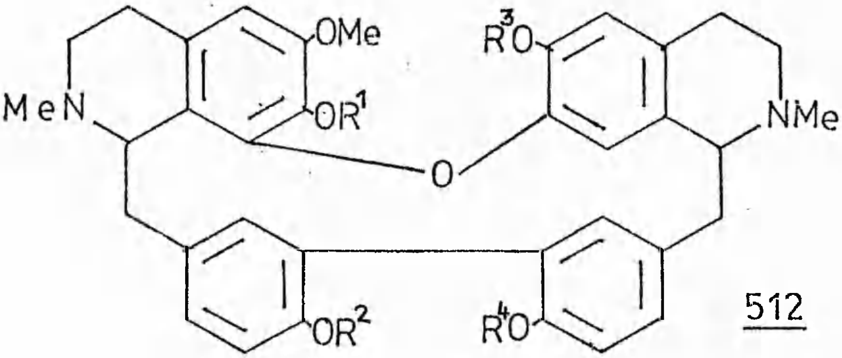
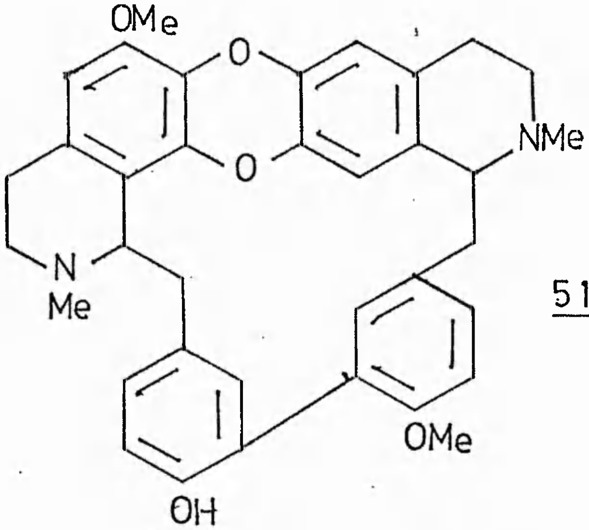


512



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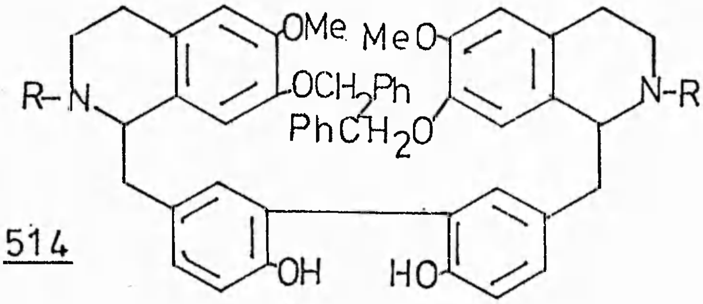


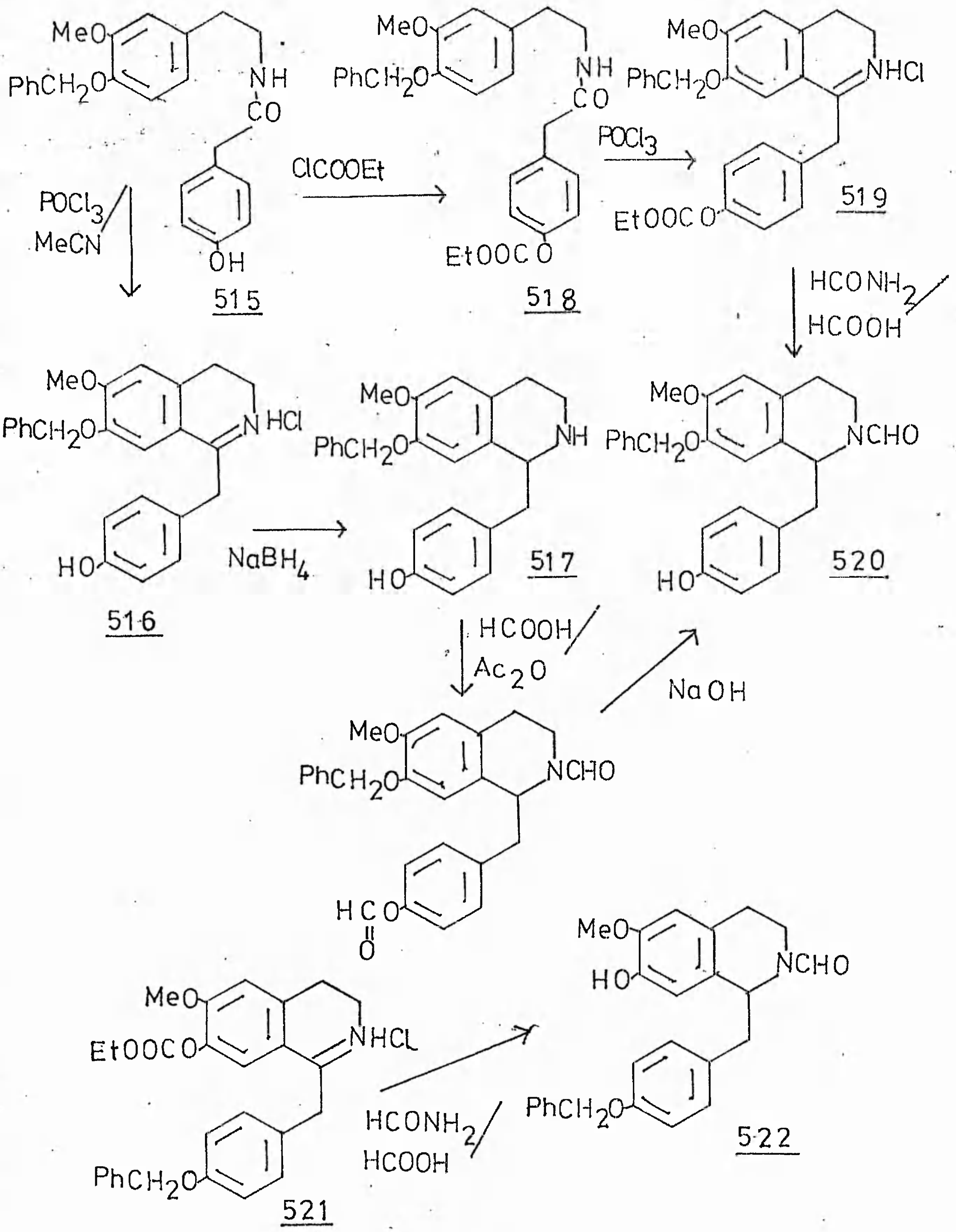
514

the present investigation this was achieved by N-formylation.

Two routes to the desired N-formylisoquinoline 520 were envisaged. The first involved preparation of 7-O-benzylcoclaurine[453] 517, which could then be O,N-diformylated, and the formate ester selectively hydrolysed. The second proceeded *via* synthesis of 7-benzyloxy-1-(4-ethoxycarbonyloxybenzyl)-6-methoxy-3,4-dihydroisoquinoline[453] 519 which, on reductive formylation[454] and subsequent hydrolysis of the ethoxycarbonyl group, would afford the desired product.

4-Benzyloxy-3-methoxy-β-phenethylamine was heated with 4-hydroxyphenylacetic acid to form N-(4-benzyloxy-3-methoxyphenethyl)-4-hydroxyphenylacetamide[453] 515, which was cyclised under the conditions of Teitel and Brossi[455] (phosphorus oxychloride in refluxing acetonitrile) to afford the hydrochloride of the dihydroisoquinoline 516. Reduction with sodium borohydride then gave the tetrahydroisoquinoline 517, but only in low yield. Formylation with a mixture of acetic anhydride and formic acid[456] produced material which was not characterised, but was immediately hydrolysed by refluxing in 2M sodium hydroxide solution to form a small amount of an amorphous product which later proved (by t.l.c. and mixed m.p.) to be identical with authentic 520.

The amide 515 was converted to the O-ethoxycarbonylderivative 518 and cyclised to the dihydroisoquinoline hydrochloride 519 as described by Kametani *et al.*[453] This compound, when subjected to reductive formylation by the method of Swan and co-workers[454] afforded, not the expected N-formyl-O-ethoxycarbonyl compound,


MeO
PhCH$_2$O
NH
CO
OH
515
POCl$_3$/MeCN
ClCOOEt
EtOOCO
518
POCl$_3$
NHCl
519
HCONH$_2$/HCOOH
N HCl
HO
516
NaBH$_4$
NH
517
NCHO
520
HCOOH/Ac$_2$O
NaOH
HCO
O
EtOOCO
NHCL
521
HCONH$_2$/HCOOH
HO
522

but a good yield of crystalline 7-benzyloxy-2-formyl-1-(4-hydroxybenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline 520, the desired final product. The structure of this compound was confirmed by high resolution mass spectrometry, analysis and infrared spectroscopy. The generality of this reaction was tested by applying it to the isomeric dihydroisoquinoline 521 (prepared by Dr. I.G.C. Coutts) and again a good yield of 1-(4-benzyloxybenzyl)-2-formyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline 522 was obtained. Although this latter compound was not oxidised in this project, it offers an alternative route to the rodiasine skeleton if intermolecular head-to-head C-O-C coupling could be effected. Methylation, debenzylation and subsequent anodic oxidation might then form a tail-to-tail biaryl link.

Synthetic organic electrochemistry including the anodic oxidation of phenols and other aromatic compounds has been adequately reviewed in three recent books.[457-9] The method used in the present case was similar to that used by Bobbitt and Hallcher[436] in the oxidation of N-carbethoxy-N-norarmepavine. Electrolysis was carried out using a graphite felt anode and a platinum foil cathode, with the anode potential being controlled by a potentiostat. The sodium salt of the phenolic amide 520 was dissolved in aqueous methanol and added to acetonitrile to give a final solution, 0.012M in amide and 0.065M in tetraethylammonium perchlorate, the electrolyte. The oxidations were carried out at constant current, the voltage being gradually increased throughout the oxidation as the substrate was consumed. The electrolysis was continued until the total charge passed was equivalent to that calculated to be required for complete oxidation of the starting material.

Two experiments were conducted, one with the current at 50mA, the other at 20mA. The products of the two oxidations were similar when examined by t.l.c. Both contained unreacted starting material, baseline material and an intermediate ill-defined spot. Attempts were made to isolate this spot by preparative t.l.c. and after two successive chromatographic separations, material which ran as a single, elongated spot was obtained. This material was amorphous, had no distinct m.p. and its infrared spectrum was similar to that of 520. A determination of its molecular weight was made to ascertain whether it was dimeric; the resulting value of 433 is not markedly greater than that of the molecular weight (387) of starting material. The oxidation product, although a single spot by t.l.c., behaved as if it were a mixture (it failed to crystallise, it did not melt cleanly etc.); although many solvent systems were tried, further work to find a system which would separate this material into its components would be desirable.

A small amount of 520 was oxidised in a two-phase system of basic ferricyanide solution and chloroform, but the product was baseline material on t.l.c. and was probably polymeric. An attempt was made to reduce some of the anodic oxidation product to the N-methyl compound, using borane-methyl sulphide complex in THF. The product, however, could not be obtained free from boron compounds.

In conclusion, the anodic oxidation of 520 gives material of different R_f value from that of starting material and determination of the molecular weight indicates that the majority of this is not dimeric. The elucidation of the precise nature of the products formed must await improvements in chromatographic purification.

EXPERIMENTAL SECTION

General

Infrared spectra were recorded using a Perkin-Elmer 137 sodium chloride spectrophotometer, calibrated with polystyrene film. Ultraviolet absorbance measurements were made using a Unicam SP 500 spectrophotometer, while the increases in absorbance with time were recorded on a Perkin-Elmer 402 u.v.-visible spectrophotometer.

Nuclear magnetic resonance spectra (^1H) were recorded using a JEOL JNM C-60 HL 60MHz spectrometer with tetramethylsilane as the internal standard in the solvent indicated.

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

Microanalyses for C, H, and N were determined by the Microanalysis Unit, University of Nottingham, and also by Butterworth Microanalytical Consultancy Ltd., Teddington, Middlesex.

High resolution mass spectrometry was carried out by the Boots Co., Nottingham. Hydrogenations were carried out using the medium pressure apparatus of Chas. W. Cook and Sons, Birmingham, while for anodic oxidations a Wenking potentiostat 70 TS1 was employed.

Thin-layer chromatography was performed using pre-spread plates (5 x 20cm^3) (Polygram SIL G/UV$_{254}$ from Camlab, Cambridge). Preparative t.l.c. (thick-layer) was performed on glass plates (20 x 20cm) coated with MN Kieselgel P/UV$_{254}$ (from Camlab, Cambridge), 60g per plate. Column chromatography was carried out using Fisons silica gel MFC (80–200 mesh).

All solvents for chromatography were redistilled. Other solvents were dried using molecular sieves (except ether, THF, and benzene which were dried over sodium).

WORK DESCRIBED IN CHAPTER TWO

Section 2.2.1

Attempted reaction of 4-benzyloxyaniline with 2-bromo-4,6-dinitrophenol

The amine (1g, 0.005 mol) and the phenol (1.3g, 0.005 mol) were dissolved in dry DMSO (7 cm^3). Copper bronze (0.5g), anhydrous potassium carbonate (1g) and a crystal of iodine were added and the mixture was heated at 100° for 3h. Water (200 cm^3) was added and the mixture extracted with chloroform (3 x 50 cm^3), the organic layer separated, dried ($MgSO_4$) and evaporated. The resulting black gum was dissolved in benzene (1 cm^3) and applied to a silica gel column (30 x 2 cm); elution with chloroform afforded a dark solid which on recrystallisation from chloroform/petroleum (b.p. 60-80°)

which on recrystallisation from chloroform/petroleum (b.p. 60-80°)

and treatment with activated charcoal furnished bright yellow plates (0.12g) of <u>4,4'-dibenzyloxyazobenzene</u> <u>368</u> m.p. 198-9°. (Found: C, 78.7; H, 5.5; N, 7.0. $C_{26}H_{22}N_2O_2$ requires C, 79.2; H, 5.5; N, 7.1%).

4-Benzyloxy-2'-nitrodiphenylamine 369

4-Benzyloxyaniline (50g, 0.25 mol) was heated with 1-bromo-2-nitrobenzene (50g, 0.25 mol) and anhydrous sodium acetate (50g) under the conditions described by McCrombie *et al*[277] to give the <u>title amine</u> <u>369</u> (44g, 55%) as a fluffy orange solid m.p. 83.5-84.5° from chloroform/ethanol. (Found: C, 71.2; H, 5.0; N, 8.6. $C_{19}H_{16}N_2O_3$ requires C, 71.3; H, 5.0; N, 8.7%) ν_{max}^{KBr}: 3320 cm^{-1} (N-H stretch). τ($CDCl_3$): 1.80, 1.93 (1H, d, exchangeable with D_2O, N-$\underline{H}$); 2.6-3.5 (13H, m, aromatics); 4.95 (2H, S, Ph$C\underline{H_2}$O).

2,4'-Dimethoxy-2'-nitrodiphenylamine 370

4-Methoxy-2-nitroaniline (3.1g, 0.02 mol), 2-bromoanisole (3.7g, 0.02 mol), copper bronze (0.5g), anhydrous potassium carbonate (5g) and pyridine (1 cm^3) were heated together at 200° for 3h. Water (100cm^3) was added and the mixture extracted with chloroform (3 x 50cm^3). The combined extracts were washed with dilute hydrochloric acid (2 x 50cm^3) and water (2 x 50cm^3), dried ($MgSO_4$) and evaporated to yield a dark red solid which was recrystallised from ethanol to give the title amine 370 (5.08g, 92%), as dark red needles m.p. 70.5-71°. (Found: C, 61.0; H, 5.2; N, 10.3. $C_{14}H_{14}N_2O_4$ requires C, 61.3; H, 5.1; N, 10.2%). ν_{max}^{KBr}: 3250 cm^{-1} (N-H stretch). τ ($CDCl_3$): 2.42, 2.46 (1H, d, exchangeable with D_2O, N-H); 2.66-3.23 (7H, m, aromatics); 6.17, 6.22 (6H, d, 2 x OCH_3).

The above compound (370) was obtained in 12% yield [after chromatography on a silica gel column, 40 x 2 cm, eluted with petroleum (b.p. 60-80°), then benzene] when 2-anisidine (2.5g, 0.02 mol), 4-bromo-3-nitroanisole (4.4g, 0.02 mol), copper bronze (0.5g), anhydrous potassium carbonate (5g) and pyridine (1cm^3) were heated together under the same conditions as above.

2,4',4''-Trimethoxy-5-nitrotriphenylamine 371

2-Methoxy-5-nitroaniline (3.9g, 0.25 mole), 4-bromoanisole (4.6g, 0.25 mole), copper bronze (0.5g) and anhydrous potassium carbonate (5g) were heated together at 200° for 4h. Work up as for 370 afforded the title amine 371 (0.68g) as orange crystals m.p. 127.5-128.5° from ethanol. (Found: C, 66.3; H, 5.4; N, 7.3.

$C_{21}H_{20}N_2O_5$ requires C, 66.3; H, 5.3; N,7.4%). τ ($CDCl_3$): 2.7-3.4 (11H, m, aromatics); 6.2 (6H, s, 2 x para OCH_3); 6.26 (3H, s, ortho OCH_3).

Section 2.2.2

N-2-Methoxyphenyl-N-4-methoxyphenylacetamide 372

2-Methoxyacetanilide[460] (6.2g, 0.04 mol), 4-bromoanisole (7.5g, 0.04 mol), anhydrous potassium carbonate (5g) and copper bronze (0.5g) were heated together at 200° for 4h. Sulphuric acid (10M, 50cm^3) was added and the mixture was refluxed for 30 min and extracted with chloroform (3 x 50cm^3). The combined extracts were washed with water (3 x 50cm^3), dried ($MgSO_4$) and evaporated to leave a black gum. This was applied to a silica gel column (40 x 2cm) and eluted with benzene to remove unreacted 4-bromoanisole. Further elution with chloroform afforded the title amide 372 (4g, 37%) as colourless prisms, m.p. 86.5-87.5° from ethanol. (Found: C, 71.1; H, 6.5; N, 5.0. $C_{16}H_{17}NO_3$ requires C, 71.0; H, 6.3; N, 5.2%). ν_{max}^{KBr}: 1660cm^{-1} (C=O). τ ($CDCl_3$): 2.64-3.37 (8H, m, aromatics); 6.22, 6.32 (6H, d, 2 x OCH_3); 8.08 (3H, s, COCH_3).

Hydrolysis of 372

The amide 372 (9g, 0.033 mol) was heated under reflux for 3h with a mixture[260] of potassium hydroxide solution (17M, 40cm^3) and ethanol (100cm^3). The mixture was extracted with chloroform (3 x 100cm^3) and the combined extracts were washed with hydrochloric acid (0.1M, 2 x 100cm^3), sodium hydroxide solution (0.1M, 2 x 100cm^3) and water (3 x 100cm^3), dried ($MgSO_4$) and evaporated. The resulting black gum was partly soluble in ether and the ether soluble fraction

(2g) was applied to a silica gel column (30 x 2.5cm); elution with benzene afforded pale yellow needles (0.18g) m.p. 70-71° from petroleum (b.p. 60-80°). This material later proved identical with 2,4′-dimethoxydiphenylamine <u>403</u> (mixed m.p. 70-71°).

<u>Section 2.2.3</u>

<u>Preparation of benzanilides</u>

These were prepared in 90-100% yield by a general method. The arylamine (0.1 mol) was dissolved in dry pyridine ($20cm^3$) and benzoyl chloride (0.1 mol) was added in one portion. After warming on the steam-bath for 30 min the mixture was poured into ice-cold hydrochloric acid (0.5M, $200cm^3$) and the resulting solid recrystallised from ethanol. The following benzanilides were prepared: N-4-methoxyphenylbenzamide, m.p. 157-9° (lit,[461] 154°); N-2-bromophenylbenzamide, m.p. 112-3° (lit,[462] 116°); N-4-benzyloxyphenylbenzamide, m.p. 234-5° (lit,[463] 235-236.5).

<u>N-2-benzyloxyphenylbenzamide 375</u>

N-2-Hydroxyphenylbenzamide was prepared by the method of Fiers-David and Kuster[280] in 94% yield, m.p. 167-8° (lit,[464] 169°). The product (21g, 0.1 mol), benzyl chloride (12.7g, 0.1 mol), anhydrous potassium carbonate (20g) and dry, redistilled acetone ($100cm^3$) were refluxed together for 48h. and the solvent then removed. The residue was poured into water ($500cm^3$) and extracted with ether ($3 \times 200cm^3$). The combined extracts were washed with potassium hydroxide solution (0.1M, $3 \times 200cm^3$) and water ($3 \times 200cm^3$), dried ($MgSO_4$) and evaporated to leave an oil which crystallised on the addition of petroleum (b.p. 60-80°). Recrystallisation from benzene afforded the <u>title amide 375</u> (21g, 70%) as colourless needles m.p. 97-8°. (Found: C, 79.3; H, 5.8; N, 4.5. $C_{20}H_{17}NO_2$ requires C, 79.2; H, 5.6; N, 4.6%) ν_{max}^{KBr}: 3470 (N-H), 1675cm^{-1} (C=O). τ($CDCl_3$): 1.5 (1H, s, exchangeable with D_2O, N-$\underline{H}$); 2.2-3.16 (14H, m, aromatics); 4.95 (2H, s, Ph$\underline{CH_2}$O).

<u>Preparation of benzimidoyl chlorides</u>

A solution of the benzanilide (0.1 mol) in thionyl chloride (40cm^3) was refluxed for 3h, and allowed to stand overnight. Petroleum (b.p. 80-100°, 50cm^3) was added and the mixture evaporated to dryness under reduced pressure. The residue was usually used in the next stage without further purification; an exception was N-4-benzyloxyphenylbenzimidoyl chloride which crystallised from petroleum (b.p. 80-100°) as yellow prisms, m.p. 118-9° (lit,[463] 112-5°). The benzimidoyl chlorides were stored over calcium chloride, as they hydrolyse rapidly in moist air.

<u>Preparation of aryl N-arylbenzimidates</u> (ref. 248)

The benzimidoyl chloride (0.02 mol) dissolved in dry benzene (20cm^3) was added dropwise to a magnetically stirred suspension/solution of the sodium salt of the phenol (0.02 mol) in absolute ethanol (20cm^3) under dry nitrogen. After being stirred for 12h the reaction mixture was poured into water (100cm^3) and extracted with ether (3 x 100cm^3). The combined extracts were washed with sodium hydroxide solution (0.5M, 3 x 200cm^3) and water (3 x 100cm^3), dried ($MgSO_4$) and evaporated. The residue could usually be crystallised, but in some cases chromatography was necessary.

2-Methoxyphenyl N-4-methoxyphenylbenzimidate 377

Colourless prisms (93%) m.p. 82-3° from benzene. (Found: C, 75.7; H, 5.6; N, 4.0. $C_{21}H_{19}NO_3$ requires C, 75.8; H, 5.4; N, 4.2%). ν_{max}^{KBr}: 1665cm^{-1} (C=N). τ($CDCl_3$): 2.2-3.5 (13H, m, aromatics); 6.19, 6.3 (6H, d, 2 x $OC\underline{H}_3$).

2-Methoxyphenyl N-4-benzyloxyphenylbenzimidate 373

Colourless prisms m.p. 118-119.5° (32%) from benzene. (Found: C, 78.9; H, 5.8; N, 3.3. $C_{27}H_{23}NO_3$ requires C, 79.2; H, 5.6; N, 3.4%). ν_{max}^{KBr}: 1660cm^{-1} (C=N). τ($CDCl_3$): 2.2-3.45 (18H, m, aromatics); 5.11 (2H, s, $PhC\underline{H}_2O$); 6.25 (3H, s, $OC\underline{H}_3$).

2-Nitrophenyl N-4-benzyloxyphenylbenzimidate 374

Bright yellow prisms m.p. 150.5-151.5 (55%) from benzene. (Found: C, 73.7; H, 4.9; N, 6.4. $C_{26}H_{20}NO_4$ requires C, 73.7; H, 4.7; N, 6.6%). ν_{max}^{KBr}: 1658cm^{-1} (C=N). τ($CDCl_3$): 2.0-3.45 (18H, m, aromatics); 5.14 (2H, s, $PhC\underline{H}_2O$).

4-Benzyloxyphenyl N-2-benzyloxyphenylbenzimidate 376

Colourless prisms m.p. 98-9° (70%) from benzene. (Found: C, 81.4; H, 5.6; N, 2.8. $C_{33}H_{27}NO_3$ requires C, 81.2; H, 5.7; N, 3.0%). ν_{max}^{KBr}: 1672cm^{-1} (C=N). τ($CDCl_3$): 2.3-3.4 (23H, m, aromatics); 5.04, 5.13 (4H, d, 2 x $PhC\underline{H}_2O$). In one experiment the residue (4g) after the work up would not crystallise and it was applied to a silica gel column (120 x 3cm); elution with benzene afforded 1,4-dibenzyloxybenzene (2.24g) as colourless prisms, m.p. 130.5-131.5 (lit,[465] 130°). (Found: C, 82.5; H, 6.3. Calc. for $C_{20}H_{18}O_2$: C, 82.8; H, 6.2%).

The rearrangement of the benzimidates

a) Rearrangement of compound 377

The benzimidate was heated under dry nitrogen using the following conditions: 200° for 3h, 250° for 3h, 280° for 1.5h, and 300° for 3 h. T.l.c. (chloroform) showed the presence of three spots with R_f values: 0.94, 0.65 (unreacted benzimidate), and 0.40. The lowest spot was major and was present in higher concentrations in the rearrangements carried out at the two higher temperatures. Preparative t.l.c. (chloroform) led to the isolation of the fast and slow spots as homogeneous, amorphous solids. The slow spot had ν_{max}^{KBr}: 1650cm^{-1} (C=O) but could not be obtained sufficiently pure for analysis. The fast spot had ν_{max}^{KBr}: 3350 (N-H) and no carbonyl absorption; it had identical R_f values in benzene and in carbon tetrachloride with those of authentic 2,4'-dimethoxydiphenylamine 403.

b) Rearrangement of compound 374

This rearrangement was more successful and the following conditions led to the isolation of the product in the yield given in parentheses; 180° for 20 min (66%), 200° for 20 min (80%) and 220° for 30 min (43%). N-4-Benzyloxyphenyl-N-2-nitrophenylbenzamide 378 was obtained as yellow crystals, m.p. 124.5-125° from benzene (charcoal). (Found: C, 73.4; H, 4.8; N, 6.5. $C_{26}H_{20}N_2O_4$ requires C, 73.6; H, 4.7; N, 6.6%). ν_{max}^{KBr}: 1672cm^{-1} (C=O). τ($CDCl_3$): 2.0-3.3 (18H, m, aromatics); 5.04 (2H, s, PhCH_2O).

c) Rearrangement of compound 376

The following conditions were used; 220° for 60 min, 270° for 30 min, 300° for 30 min, 310° for 60 min, 330° for 20 min. T.l.c. (chloroform) of the products generally showed the presence of five spots with R_f values: 0.95 (blue), 0.79 (minor), 0.67 (unreacted benzimidate), 0.57 (major), and 0.38 (minor). Chromatography on silica gel columns with elution by chloroform afforded the 0.95 and the 0.57 spots as virtually homogeneous gums. The faster-running of these materials was unstable, darkening and becoming insoluble when kept in air. Its chromatographic behaviour was similar to that of the fast-running material from the rearrangement of 377. Its infrared spectrum showed a peak at $3250 cm^{-1}$ and no carbonyl absorption. The material ($R_f = 0.57$) was a hard gum which would not crystallise from any solvent. An attempt at sublimation (100-130°, 0.5 mm Hg) led to the isolation of a solid which was obtained as colourless crystals from ethyl acetate/petroleum (b.p. 80-100°), m.p. 172.5-173.5, mixed m.p. with hydroquinone 172-3°. (Found: C, 65.1; H, 5.6. Calc. for $C_6H_6O_2$: C, 65.3; H, 5.5%). The infrared spectrum was superimposable on that of authentic hydroquinone.

Section 2.3

Preparation of N-aryltoluene-4-sulphonamides

These were prepared in 90-100% yield by a general method. The arylamine (0.1 mol) was dissolved in dry pyridine ($20 cm^3$) and toluene-4-sulphonylchloride (0.1 mol) was added. After warming on the steam-bath for 30 min the reaction mixture was poured into ice-cold hydrochloric acid (1M, 500cm^3) and the resulting solid crystallised from ethanol. The sulphonamides which were prepared are shown in Table IV.

TABLE IV

The preparation of secondary sulphonamides $RC_6H_4 \cdot NH \cdot SO_2 \cdot C_6H_4Me$-4

R	m.p.	lit m.p.	(ref)
2-MeO	126-7°	127°	(466)
3-MeO	65-6°	68°	(467)
4-MeO	113-4°	114°	(468)
H	101-2°	103°	(469)
4-Me	116-7°	118°	(469)
3,4-diMe	143-4°	144.5-145°	(471)
2-PhCH$_2$O	98-9°	99.5-100°	(472)
4-PhCH$_2$O	156-7°	157°	(473)
2-NO$_2$	111-2°	111-3°	(474)
4-NO$_2$	190-1°	189-90°	(475)

Reaction of N-arylsulphonamides with aryl bromides

The secondary sulphonamide (0.01 mol), aryl bromide (0.012 mol), anhydrous potassium carbonate (0.012 mol) and copper bronze (2g) were mixed together and heated for 24h under an air condenser at 180°; during the heating the mixture was swirled periodically, when carbon dioxide was evolved. In the case of aryl halides bearing a nitro substituent the heating time was reduced to 6h. At the end of the heating time the flask was fitted with a distillation head and the excess aryl bromide was distilled off at 180° and 1mm Hg. The residue was digested with water and extracted with chloroform (3 x 50cm^3). The combined extracts, after filtration through celite, were washed with sodium hydroxide solution (1M, 100cm^3 portions) until secondary sulphonamide was no longer extracted, then with water (2 x 100cm^3) and dried (Na_2SO_4). Evaporation gave a black gum or solid which was dissolved in benzene, treated repeatedly with activated charcoal and crystallised with the addition of petroleum (b.p. 80-100°). N,N-Diphenyltoluene-4-sulphonamide crystallised from ethanol as colourless prisms, m.p. 141-2° (lit,[476] 141°).

N-2-Methoxyphenyl-N-4-methoxyphenyltoluene-4-sulphonamide 379

Colourless prisms, m.p. 106-106.5° from ethanol. (Found: C, 65.7; H, 5.5; N, 3.6. $C_{21}H_{21}NO_4S$ requires C, 65.8; H, 5.5; N, 3.7%). $\tau(CDCl_3)$: 2.48-3.38 (12H, m, aromatics); 6.31, 6.38 (6H, d, 2 x $OC\underline{H_3}$); 7.64 (3H, s, $C_6H_4C\underline{H_3}$).

N,N-di(4-methoxyphenyl)-toluene-4-sulphonamide 380

Colourless prisms, m.p. 129-130° from ethanol. (Found: C, 65.5; H, 5.5; N, 3.5. $C_{21}H_{21}NO_4S$ requires C, 65.8; H, 5.5, N, 3.7%). $\tau(CDCl_3)$: 2.45-3.38 (12H, m, aromatics): 6.33 (6H, s 2 x $OC\underline{H_3}$); 7.67 (3H, s, $C_6H_4C\underline{H_3}$).

N-4-Methoxyphenyl-N-4-tolytoluene-4-sulphonamide 381

Colourless prisms, m.p. 96-7° from ethanol. (Found: C, 68.9; H, 5.5; N, 3.6. $C_{21}H_{21}NO_3S$ requires C, 68.7; H, 5.7; N, 3.8%). $\tau(CDCl_3)$: 2.4-3.35 (12H, m, aromatics); 6.27 (3H, s, $OC\underline{H_3}$); 7.61, 7.73 (6H, d, 2 x $C_6H_4C\underline{H_3}$).

N-4-Methoxyphenyl-N-phenyltoluene-4-sulphonamide 382

Colourless prisms, m.p. 92.5-93° from ethanol. (Found: C, 68.2; H, 5.2; N, 3.9. $C_{20}H_{19}NO_3S$ requires C, 68.0; H, 5.4; N, 4.0%). $\tau(CDCl_3)$: 2.4-3.36 (13H, m, aromatics); 6.3 (3H, s, $OC\underline{H_3}$); 7.64 (3H, s, $C_6H_4C\underline{H_3}$).

N-Phenyl-N-4-tolyltoluene-4-sulphonamide 383

Colourless prisms, m.p. 120-1° from ethanol. (Found: C, 70.9; H, 5.6; N, 4.0. $C_{20}H_{19}NO_2S$ requires C, 71.1; H, 5.6; N, 4.2%). $\tau(CDCl_3)$: 2.37-2.9 (13H, m, aromatics); 7.58; 7.7 (6H, d, 2 x $C_6H_4C\underline{H}_3$).

N,N-Di-(4-tolyl)-toluene-4-sulphonamide 384

Colourless prisms, m.p. 141-2° from ethanol. (Found: C, 71.5; H, 6.0; N, 3.9. $C_{21}H_{21}NO_2S$ requires C, 71.8; H, 6.0; N, 4.0%). $\tau(CDCl_3)$: 2.38-2.91(12H, quartet, aromatics); 7.60 (3H, s, $SO_2C_6H_4C\underline{H}_3$); 7.72 (6H, s, 2 x $NC_6H_4C\underline{H}_3$).

N-4-Biphenyl-N-2-methoxyphenyltoluene-4-sulphonamide 385

Colourless prisms, m.p. 169-170° from chloroform/ethanol. (Found: C, 72.3; H, 5.4; N, 3.2. $C_{26}H_{23}NO_3S$ requires C, 72.6; H, 5.4; N, 3.3%). $\tau(CDCl_3)$: 2.36-3.25 (17H, m, aromatics); 6.37 (3H, s, $OC\underline{H}_3$); 7.64 (3H, s, $C_6H_4C\underline{H}_3$).

N-4-Chlorophenyl-N-2-methoxyphenyltoluene-4-sulphonamide 386

Colourless prisms, m.p. 122-3° from chloroform/ethanol. (Found: C, 61.8; H, 4.8; N, 3.5. $C_{20}H_{18}NO_3SCl$ requires C, 62.0; H, 4.6; N, 3.6%). $\tau(CDCl_3)$: 2.32-3.25 (12H, m, aromatics); 6.88 (3H, s, $OC\underline{H}_3$); 7.62 (3H, s, $C_6H_4C\underline{H}_3$).

N-2-methoxyphenyl-N-4-methoxy-2-methylphenyltoluene-4-sulphonamide 387

Colourless prisms, m.p. 136-7° from chloroform/ethanol. (Found: C, 66.4; H, 5.7; N, 3.4. $C_{22}H_{23}NO_4S$ requires C, 66.5; H, 5.8; N, 3.5%). $\tau(CDCl_3)$: 2.3-3.42 (11H, m, aromatics); 6.24, 6.33 (6H, d, 2 x $OC\underline{H}_3$); 7.6 (3H, s, $SO_2C_6H_4C\underline{H}_3$); 7.88 (3H, s, $NC_6H_3C\underline{H}_3$).

N-2-Methoxyphenyl-N-3,4-dimethylphenyltoluene-4-sulphonamide 388

Colourless prisms, m.p. 135-6° from chloroform/ethanol. (Found: C, 69.2; H, 6.2; N, 3.5. $C_{22}H_{23}NO_3S$ requires C, 69.3; H, 6.0; N, 3.7%). τ($CDCl_3$): 2.33-3.28 (11H, m, aromatics); 6.41 (3H, s, $OC\underline{H}_3$); 7.67 (3H, s, $SO_2C_6H_4C\underline{H}_3$); 7.87 (6H, s, 2 x $NC_6H_3C\underline{H}_3$).

N-4-Benzyloxyphenyl-N-4-methoxyphenyltoluene-4-sulphonamide 389

Colourless prisms, m.p. 147-8° from chloroform/ethanol. (Found: C, 69.4; H, 5.5; N, 2.8. $C_{27}H_{25}NO_4S$ requires C, 69.1; H, 5.3; N, 3.0%). τ($CDCl_3$): 2.4-3.34 (17H, m, aromatics); 5.04 (2H, s, $PhC\underline{H}_2O$); 6.28 (3H, s, $OC\underline{H}_3$); 7.63 (3H, s, $C_6H_4C\underline{H}_3$).

N-2-Benzyloxyphenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 390

Colourless prisms, m.p. 134-5° from benzene/petroleum (b.p. 80-100°). (Found: C, 74.0; H, 5.7; N, 2.3. $C_{33}H_{29}NO_4S$ requires C, 74.1; H, 5.4; N, 2.6%). τ($CDCl_3$): 2.36-3.33 (22H, m, aromatics); 5.04, 5.09 (4H, d, 2 x $PhC\underline{H}_2O$); 7.66 (3H, s, $C_6H_4C\underline{H}_3$).

N-4-Nitrophenyl-N-phenyltoluene-4-sulphonamide 391

Yellow needles, m.p. 161-2° from ethanol. (Found: C, 62.0; H, 4.3; N, 7.5. $C_{19}H_{16}N_2O_4S$ requires C, 62.0; H, 4.3; N, 7.6%). τ($CDCl_3$): 1.86-2.84 (13H, m, aromatics); 7.62 (3H, s, $C_6H_4C\underline{H}_3$).

N-4-Nitrophenyl-N-4-tolyltoluene-4-sulphonamide 392

Yellow prisms, m.p. 148-9° from ethanol. (Found: C, 62.5; H, 4.7; N, 7.3. $C_{20}H_{18}N_2O_4S$ requires C, 62.8; H, 4.7; N, 7.3%). τ($CDCl_3$): 1.84-2.9 (12H, m, aromatics); 7.57, 7.63 (6H, d, 2 x $C_6H_4C\underline{H}_3$).

N-4-Methoxyphenyl-N-4-nitrophenyltoluene-4-sulphonamide 393

Yellow needles, m.p. 100-101° from ethanol. (Found: C, 60.1; H, 4.5; N, 6.9. $C_{20}H_{18}N_2O_5S$ requires C, 60.3, H, 4.5; N, 7.0%). $\tau(CDCl_3)$: 1.88-3.27 (12H, m, aromatics); 6.23 (3H, s, $OC\underline{H}_3$); 7.63 (3H, s, $C_6H_4C\underline{H}_3$).

N-3-Methoxyphenyl-N-4-nitrophenyltoluene-4-sulphonamide 394

Yellow needles, m.p. 103-4° from ethanol. (Found: C, 60.1; H, 4.8; N, 6.8. $C_{20}H_{18}N_2O_5S$ requires C, 60.3; H, 4.5; N, 7.0%). $\tau(CDCl_3)$: 1.80-3.25 (12H, m, aromatics); 6.75 (3H, s, $OC\underline{H}_3$); 7.59 (3H, s, $C_6H_4C\underline{H}_3$).

N-4-Benzyloxyphenyl-N-4-nitrophenyltoluene-4-sulphonamide 395

Yellow needles, m.p. 132-3° from benzene. (Found: C, 65.5; H, 4.7; N, 5.7. $C_{26}H_{22}N_2O_5S$ requires C, 65.8; H, 4.6; N, 5.9%). $\tau(CDCl_3)$: 1.80-2.97 (17H, m, aromatics); 4.94 (2H, s, $PhC\underline{H}_2O$); 7.57 (3H, s, $C_6H_4C\underline{H}_3$).

N-4-Benzyloxyphenyl-N-2-nitrophenyltoluene-4-sulphonamide 396

Yellow needles, m.p. 147-8° from ethanol. (Found: C, 65.5; H, 4.8; N, 5.8. $C_{26}H_{22}N_2O_5S$ requires C, 65.8; H, 4.6; N, 5.9%). $\tau(CDCl_3)$: 2.1-3.25 (17H, m, aromatics); 5.02 (2H, s, $PhC\underline{H}_2O$); 7.58 (3H, s, $C_6H_4C\underline{H}_3$).

N-3,4-Dimethylphenyl-N-2-nitrophenyltoluene-4-sulphonamide 397

Yellow prisms, m.p. 191.5-192.5° from benzene. (Found: C, 63.7; H, 5.2; N, 7.1. $C_{21}H_{20}N_2O_5S$ requires C, 63.6; H, 5.1; N, 7.1%). $\tau(CDCl_3)$: 2.11-3.0 (11H, m, aromatics); 7.6 (3H, s, $SO_2C_6H_4C\underline{H}_3$); 7.82 (6H, s, 2 x $NC_6H_3C\underline{H}_3$).

Reaction of sulphonamides with aryl bromides under the conditions of Bacon and Karim[290]

The secondary sulphonamide (0.01 mol) and a dispersion of sodium hydride in oil (0.01 mol) were added to dry DMF; after the evolution of hydrogen had ceased the mixture was refluxed for 12h. The aryl bromide (0.01 mol) and copper (II) iodide (0.01 mol) were then added and refluxing was continued for a further 24h. Moisture was rigorously excluded throughout. The mixture was then poured into water ($200cm^3$) and extracted with chloroform ($3 \times 100cm^3$). The combined extracts were washed exhaustively with sodium hydroxide solution (1M, $100cm^3$ portions), water ($2 \times 200cm^3$), dried (Na_2SO_4) and evaporated. The products, after crystallisation, were identical by t.l.c. ($CHCl_3$), m.p., mixed m.p. and infrared spectra with the corresponding compounds prepared by the Goldberg-type route.

N-Aryl-4-nitrobenzenesulphonamides

These were prepared as described for the toluene-4-sulphonamides but using 4-nitrobenzenesulphonyl chloride.

N-Phenyl-4-nitrobenzenesulphonamide was obtained as yellow needles, m.p. 169-70° from ethanol (lit[477] 168.7-169.7°). (Found: C, 51.5, H, 3.7; N, 9.8. Calc. for $C_{12}H_{10}N_2O_4S$: C, 51.8; H, 3.6; N, 10.0%). N-4-Methoxyphenyl-4-nitrobenzenesulphonamide was obtained as yellow needles from ethanol, m.p. 182-3° (lit[477] 182-3°). (Found: C, 50.4; H, 4.1; N, 8.9. Calc. for $C_{13}H_{12}N_2O_5S$: C, 50.6; H, 3.9: N, 9.1%).

Preparation of N,N-diaryl-4-nitrobenzenesulphonamides

These were prepared using the Goldberg-type procedure described for the N,N-diaryltoluene-4-sulphonamides.

N-4-Methoxyphenyl-N-phenyl-4-nitrobenzenesulphonamide 398.

Pale yellow needles, m.p. 151-2° from ethanol. (Found: C, 59.2; H, 4.0; N, 7.5. $C_{19}H_{16}N_2O_5S$ requires C, 59.3; H, 4.2; N, 7.3%). τ(CDCl$_3$): 1.60-3.27 (13H, m, aromatics); 6.19 (3H, s, OC$\underline{H_3}$).

N,N-Di(4-methoxyphenyl)-4-nitrobenzenesulphonamide 399

Pale yellow needles, m.p. 141-2° from ethanol. (Found: C, 57.7; H, 4.3; N, 6.7. $C_{20}H_{18}N_2O_6S$ requires C, 58.0; H, 4.3; N, 6.8%). τ(CDCl$_3$): 1.66-3.33 (12H, m, aromatics); 6.26 (6H, s, 2 x OC$\underline{H_3}$).

Reaction of N-2- and 4-nitrophenyltoluene-4-sulphonamides with aryl bromides

The reactions were carried out by the Goldberg-type procedure. The residue left after removal of the unreacted secondary sulphonamide and the excess aryl bromide was shown by t.l.c. (CHCl$_3$) to contain, in the case of the 2-nitro compound, only tar, and in the case of the 4-nitro compound a spot which had a different R_f value from that of the expected tertiary sulphonamide. Chromatography on a silica gel column (50 x 2cm) and elution with chloroform led to the isolation of 4-nitrotriphenylamine 400 in 7% yield which crystallised from ethanol as shiny yellow plates, m.p. 142-3° (lit[478] 144°). (Found: C, 74.4; H, 4.8; N, 9.8. Calc. for $C_{18}H_{14}N_2O_2$: C, 74.5; H, 4.8; N, 9.7%).

4,4′-Dimethyl-4″-nitrotriphenylamine 401 was obtained in 6% yield as yellow plates, m.p. 162-3° from ethanol. (Found: C, 75.1;

H, 5.7; N, 8.8. $C_{20}H_{18}N_2O_2$ requires C, 75.4; H, 5.7; N, 8.8%). τ($CDCl_3$): 1.97-3.3 (12H, s and quartet, aromatics); 7.68 (6H, s, 2 x $C_6H_4C\underline{H_3}$).

4,4',4"-Trinitrotriphenylamine 402 was isolated in 4% yield as orange crystals from aqueous DMF, m.p. > 300°. (402 was insoluble in all common organic solvents). (Found: C, 61.1; H, 3.4; N, 15.5. $C_{18}H_{12}N_4O_6$ requires C, 61.6; H, 3.4; N, 16.0%).

2,4'-Dimethoxydiphenylamine 403

The tertiary sulphonamide 379 (3.8g, 0.01 mol) was added to a solution of sodium (1.05g, 0.045 mol) in 3-methylbutan-1-ol (16g, 0.18 mol) and the mixture was heated under reflux for 24h. Hydrochloric acid (0.5M, 40cm^3) was added, the mixture extracted with ether (3 x 50cm^3). The combined extracts were washed with water (2 x 100cm^3), dried (Na_2SO_4) and evaporated to leave a dark gum. This was extracted thoroughly with boiling hexane; concentration of the extracts afforded the title amine 403 (1.6g, 68%) as colourless needles, m.p. 71-2° from hexane. (Found: C, 72.8; H, 6.7; N, 6.2. $C_{14}H_{15}NO_2$ requires C, 73.4; H, 6.6; N, 6.1%). τ($CDCl_3$): 2.8-3.33 (8H, m, aromatics); 4.1 (1H, broad s, exchangeable with D_2O, N-$\underline{H}$); 6.16, 6.26 (6H, d, 2 x $OC\underline{H_3}$).

Acylation of compound 403

2,4'-Dimethoxydiphenylamine (1g, 0.004 mol), anhydrous zinc chloride (1g) and acetic anhydride (10cm^3) were stirred (magnetically) together in a stoppered flask for 60h. Water (100cm^3) was then added and the resulting brown gum extracted with dichloromethane (50cm^3). The organic layer was washed with water (3 x 100cm^3), dried ($MgSO_4$) and evaporated to afford N-2-methoxyphenyl-N-4-methoxyphenylacetamide (1.1g, 85%) which crystallised from ethanol as colourless prisms, m.p. 85-6°, mixed m.p. with 372 85-6°. Its infrared spectrum was superimposable on that of 372.

Similar treatment of 403 with benzoyl chloride afforded a gum which t.l.c. ($CHCl_3$) showed to contain a spot which had the same R_f (4.0) as the slow spot from the rearrangement of the benzimidate 377.

Section 2.4.1

Attempted demethylation of 379

a) The tertiary sulphonamide (2g, 0.005 mol) and anhydrous aluminium chloride (4g) in dry benzene ($15cm^3$) were heated at 110° for 1h. Crushed ice (50g) and hydrochloric acid (1M, $50cm^3$) were added to the black residue and the resulting solid filtered, extracted with sodium hydroxide solution (1M, $20cm^3$) and reacidified to afford a black solid (0.8g).

b) The sulphonamide (0.5g) was suspended in a solution of hydrogen bromide in glacial acetic acid (45% w/v, $10cm^3$) and heated at 100° for 3h. Addition of water ($100cm^3$) gave a black material which was reprecipitated from base to give 0.05g of a black solid.

c) The sulphonamide (5g) was heated with pyridine hydrochloride (25g) at 180° for 1h. Water ($300cm^3$) was added and the resulting material reprecipitated from base to produce again a black solid (4.7g).

d) The sulphonamide (29.4g, 0.074 mol) in dry dichloromethane ($600cm^3$) was added over 30 min to boron tribromide (65.5g, 0.26 mol) in dry dichloromethane ($125cm^3$) and the mixture stirred magnetically for 24h at 0-4°. The solution was poured cautiously into water ($600cm^3$) and the resulting solid reprecipitated from sodium hydroxide solution as above to yield a black solid (23g).

The black solids obtained from the above experiments were similar; m.p.s varied from 180-250° but were not sharp. Their infrared spectra showed hydroxyl absorptions at $3400cm^{-1}$. T.l.c. ($CHCl_3$/5% MeOH) showed the presence of four components in approximately equal amounts but column or preparative thin-layer chromatography did not lead to any separation. Repeated treatment with activated charcoal of a solution of the black solid in ethanol did not remove the colour. Treatment of a portion of the black solid with acetic anhydride and glacial acetic acid afforded a clear brown glass which showed the carbonyl absorption ($1720cm^{-1}$) of a phenyl acetate. Column chromatography and sublimation both failed to effect purification of this material.

Section 2.4.2

1 Methoxymethoxy-2-nitrobenzene 404

2-Nitrophenol (28g, 0.2 mol) was added to a solution of sodium (4.6g, 0.2 mol) in absolute ethanol ($100cm^3$) and the solvent removed under reduced pressure. The resulting red solid was suspended in dry benzene ($100cm^3$) containing chloromethyl methyl ether (16.2g, 0.2 mol) and the mixture heated under reflux for 3h. The benzene solution was washed with sodium hydroxide solution (1M, 2 x $100cm^3$), water (2 x $100cm^3$), dried ($MgSO_4$) and evaporated to afford a yellow oil which solidified to produce the title ether 404 (33.8g, 94%) as green-yellow prisms m.p. 35-6° from petroleum (b.p.60-80°). (Found: C, 52.3; H, 4.9; N, 7.6. $C_8H_9NO_4$ requires C, 52.4; H, 4.9; N, 7.7%). τ($CDCl_3$): 2.26-3.12 (4H, m, aromatics); 4.73 (2H, s, $OC\underline{H_2}O$); 6.46 (3H, s, $OC\underline{H_3}$).

2-Methoxymethoxyaniline

The nitro compound 404 (36.6g, 0.2 mol) was dissolved in ethanol (150cm^3) and added to a solution of sodium dithionite (100g) in water (150cm^3). The mixture was refluxed for 2h and then distilled until 200cm^3 of distillate was collected. The residue was extracted with ether (3 x 100cm^3) and the combined extracts washed with water (2 x 100cm^3), dried ($MgSO_4$) and evaporated to leave a yellow crystalline solid (16g) which rapidly changed to a dark liquid. Distillation of the dark liquid gave a colourless liquid (b.p. 74-7°, 0.8mm Hg) which quickly solidified but darkened after a few hours. ν_{max}^{KBr}: 3350, 3280 (NH_2).

The reduction was also carried out catalytically. The nitro compound (13.5g, 0.074 mol) was dissolved in ethanol (100cm^3) and palladium on charcoal (5%, 1g) added. The mixture was shaken under hydrogen (50 p.s.i.) until uptake of gas ceased, filtered and evaporated to leave the amine (11.1g, 98%) as a yellow crystalline solid which rapidly darkened.

N-2-Methoxymethoxyphenyltoluene-4-sulphonamide 405

Freshly prepared 2-methoxymethoxyaniline was treated with toluene-4-sulphonyl chloride in pyridine to afford the title sulphonamide 405 in 88% yield as colourless plates, m.p. 82.5-83.5° from benzene/petroleum (b.p. 80-100°). (Found: C, 58.6;

H, 5.6; N, 4.4. $C_{15}H_{17}NO_4S$ requires C, 58.7; H, 5.5; N, 4.6%) $\tau(CDCl_3)$: 2.72 (1H, s, exchangeable with D_2O, N-$\underline{H}$); 2.28-3.13 (8H, m, aromatics); 5.07 (2H, s, $OC\underline{H_2}O$); 6.22 (3H, s, $OC\underline{H_3}$); 7.67 (3H, s, $C_6H_4C\underline{H_3}$).

1-Bromo-4-methoxymethoxybenzene 406

4-Bromophenol (58g, 0.33 mol) was added to a solution of sodium (7.6g, 0.33 mol) in dry methanol (200 cm^3) and the solvent was then removed under reduced pressure. The resulting solid was dissolved in dry DMF (150cm^3) containing chloromethyl methyl ether (27g, 0.33 mol) and the solution was heated at 120° for 1h, poured into sodium hydroxide solution (1M, 500cm^3) and extracted with ether (3 x 300cm^3). The combined extracts were washed with sodium hydroxide solution (1M, 2 x 400cm^3), water (2 x 500cm^3) and dried ($MgSO_4$). Evaporation gave a colourless liquid, distillation of which afforded the *title ether* 406 (33g, 46%) b.p. 88-9°, 0.9mm Hg. (Found: C, 44.1; H, 4.4. $C_8H_9O_2Br$ requires C, 44.3; H, 4.2.). $\tau(CDCl_3)$: 2.7, 2.84, 3.28, 3.32 (4H, quartet, aromatics); 5.03 (2H, s, $OC\underline{H_2}O$); 6.68 (3H, s, $OC\underline{H_3}$).

Section 2.4.3

Preparation of N-2-benzyloxyphenyltoluene-4-sulphonamide

a) 2-Aminophenol (55g, 0.5 mol) and toluene-4-sulphonyl chloride (95g, 0.5 mol) were added to phosphate buffer (2M, pH = 7.0, 200cm^3) and the mixture warmed for 30 min on the steam-bath. Hydrochloric acid (4M, 1dm^3) was then added and the resulting solid was recrystallised from aqueous ethanol to afford N-2-hydroxyphenyltoluene-4-sulphonamide (110g, 84%)as colourless crystals, m.p. 139-40° (lit[479] 138-9°).

The above sulphonamide (53g, 0.2 mol), benzyl chloride (25.5g, 0.2 mol) and anhydrous potassium carbonate (30g) were added to dry acetone (200cm^3) and the mixture was refluxed for 24h with mechanical stirring. The mixture was evaporated, poured into water (300cm^3) and extracted with chloroform (3 x 100cm^3). The combined extracts were washed with saturated sodium carbonate solution (3 x 100cm^3), water (2 x 100cm^3), dried (MgSO$_4$) and evaporated to yield a brown solid which was recrystallised from ethanol to afford N-benzyl-N-2-hydroxyphenyltoluene-4 sulphonamide 407 (53g, 76%) as colourless prisms, m.p. 158-9°. (Found: C, 67.9; H, 5.5; N, 4.0. $C_{20}H_{19}NO_3S$ requires C, 68.0; H, 5.4; N, 4.0%) ν^{KBr}_{max}: 3460cm^{-1} (O-H). τ(CDCl$_3$): 2.38-3.68 (14H, m, aromatics and O-$\underline{H}$); 5.36 (2H, s, PhC$\underline{H_2}$N); 7.68 (3H, s, $C_6H_4C\underline{H_3}$).

b) 2-Benzyloxyaniline was prepared from 2-hydroxyacetanilide by the method of Ek and Witkop[281] in 88% yield and tosylated to afford N-2-benzyloxyphenyltoluene-4-sulphonamide as colourless plates, m.p. 98-9° from chloroform/ethanol (lit[472] 99.5-100°). 1-Benzyloxy-4-bromobenzene was obtained in 95% yield by benzylating 4-bromophenol in acetone and recrystallised from petroleum (b.p. 60-80°) as colourless prisms, m.p. 62-3° (lit[480] 64-5°).

N-2-Benzyloxyphenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 390 (4g, 0.0075 mol) and palladised charcoal (5%, 1g) were added to 1,4-dioxan (200cm^3) containing concentrated hydrochloric acid (5cm^3) and the mixture shaken under hydrogen (50 p.s.i.) at 80°; further amounts (0.5g) of catalyst were added when the rate of uptake of gas slowed down. When hydrogen ceased to be absorbed the mixture was filtered and evaporated to leave a clear gum which was dissolved in sodium hydroxide solution (1M, 200cm^3); the solution was filtered and acidified with dilute hydrochloric acid. The resulting white precipitate was recrystallised from ethyl acetate/petroleum (b.p. 80-100°) to afford N-2-hydroxyphenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 408 (2g, 76%) as colourless prisms, m.p. 176-7°. (Found: C, 63.8; H, 5.0; N, 3.7. $C_{19}H_{17}NO_4S$ requires C, 64.1; H, 4.8; N, 3.9%). ν_{max}^{KBr}: 3420, 3360cm^{-1} (2 x O-H). τ(d_6DMSO):2.56-3.51 (12H, m, aromatics); 7.57 (3H, s, $C_6H_4CH_3$). (If ethanol was used to recrystallise 408 a product melting at 134-5° was obtained; n.m.r. spectroscopy showed this to be 408 with half a molecule of ethanol of crystallisation.)

Section 2.5

N-4-Benzyloxyphenyl-N-2-nitrophenyltoluene-4-sulphonamide 396 (30g, 0.063 mol) and palladised charcoal (5%, 2g) were added to 1,4-dioxan (300cm^3) containing concentrated hydrochloric acid (10cm^3), and the mixture was shaken under hydrogen until the uptake of gas ceased. Filtration and evaporation of the mixture afforded a gum which, on the addition of water (100cm^3) and ammonia solution (d0.88, 20cm^2), solidified. Recrystallisation from ethanol produced N-2-aminophenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 410 (18g, 80%) as colourless prisms, m.p. 200.5-201.5°. (Found: C, 64.3; H, 5.3; N, 7.9. $C_{19}H_{18}N_2O_3S$ requires C, 64.6; H, 5.1; N, 7.9%) ν_{max}^{KBr}: 3510 (O-H); 3430, 3370cm^{-1} (NH_2). τ(d_6DMSO): 2.57-3.63 (12H, m, aromatics); 7.66 (3H, s, $C_6H_4CH_3$).

N-4-Benzyloxyphenyl-N-2-nitrophenyltoluene-4-sulphonamide 396 (20g, 0.042mol) was dissolved in dry benzene (200cm^3) and the resulting solution added dropwise under dry nitrogen to a magnetically stirred suspension of sodium borohydride (10g) and palladised charcoal (5%, 1g) in absolute ethanol (100cm^3). Stirring under nitrogen was continued for 3h and water (500cm^3) and ethyl acetate (500cm^3) were then added. The organic layer was washed with water (500cm^3), dried (Na_2SO_4), and evaporated to give a white solid. Recrystallisation from chloroform/cyclohexane afforded N-2-aminophenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 411 (16.8g, 90%) as colourless prisms, m.p. 152-152.5°. (Found: C, 70.2; H, 5.6; N, 6.1. $C_{26}H_{24}N_2O_3S$ requires C, 70.3; H, 5.4; N, 6.3%). ν_{max}^{KBr}: 3530, 3430cm^{-1} (NH_2). τ($CDCl_3$): 2.37-3.53 (17H, m, aromatics); 5.06 (2H, s, $PhCH_2O$); 5.93 (2H, broad s, exchangeable with D_2O, NH_2); 7.64 (3H, s, $C_6H_4CH_3$).

The aminosulphonamide 411 was treated with benzoyl chloride in pyridine to afford N-2-benzamidophenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 412 (67%) as colourless crystals, m.p. 170-170.5° from ethanol. (Found: C, 72.4; H, 5.2; N, 5.0. $C_{33}H_{28}N_2O_4S$ requires C, 72.3; H, 5.1; N, 5.1%). ν_{max}^{KBr}: 3390 (N-H); 1675cm^{-1} (C=O). τ($CDCl_3$): 2.37-3.28 (22H, m, aromatics); 5.07 (2H, s, $PhCH_2O$). 7.62 (3H, s, $C_6H_4CH_3$).

Compound 411 was treated with toluene-4-sulphonyl chloride in pyridine to produce N-4-benzyloxyphenyl-N-2-(toluene-4-sulphonamido)-phenyltoluene-4-sulphonamide 413 (97%) as colourless crystals, m.p.

155-6° from benzene/petroleum (b.p. 80-100°). (Found: C, 66.5; H, 5.1; N, 4.8. $C_{33}H_{30}N_2O_5S_2$ requires C, 66.2; H, 5.0; N, 4.7%). ν_{max}^{KBr}: 3360cm^{-1} (N-H). τ($CDCl_3$): 2.22-3.33 (21H, m, aromatics); 5.01 (2H, s, $PhC\underline{H_2}O$); 7.61, 7.72 (6H, d, 2 x $C_6H_4C\underline{H_3}$); 8.2 (1H, s, exchangeable with D_2O, N$\underline{H}$).

Compound 411 (12g, 0.027 mol), benzyl chloride (4g, 0.03 mol), anhydrous potassium carbonate (6g), and dry DMF (20cm^3) were heated together under reflux for 2h. Water (200cm^3) was added and the mixture extracted with chloroform (3 x 100cm^3). The combined extracts were washed with water (2 x 100cm^3), dried (Na_2SO_4), and evaporated to leave a white solid. Recrystallisation from chloroform/ethanol afforded N-2-benzylaminophenyl-N-4-benzyloxyphenyltoluene-4-sulphonamide 414 (68%) as colourless crystals, m.p. 166-166.5°. (Found: C, 74.0; H, 5.8; N, 5.3. $C_{33}H_{30}N_2O_3S$ requires C, 74.1; H, 5.6; N, 5.2%). ν_{max}^{KBr}: 3420cm^{-1} (N-H). τ($CDCl_3$): 2.37-3.50 (22H, m, aromatics); 5.0 (2H, s, $PhC\underline{H_2}O$); 5.68 (2H, s, $PhC\underline{H_2}N$); 7.62 (3H, s, $C_6H_4C\underline{H_3}$); 8.47 (1H, s, exchangeable with D_2O, N$\underline{H}$).

The aminosulphonamide 411 (13.2g, 0.03 mol), 1-fluoro-2,4-dinitrobenzene (8g, 0.043 mol), anhydrous potassium carbonate (6g), and copper bronze (2g) were mixed together and heated at 160° for 24h. The mixture was digested with water (100cm^3) and extracted with chloroform (3 x 100cm^3). The combined extracts were washed with water (2 x 100cm^3), dried (Na_2SO_4), and evaporated to give a black gum which was applied to a silica gel column (40 x 5cm). Elution with benzene afforded first the aryl fluoride, then the sulphonamide

411, and finally a yellow solid which on recrystallisation from chloroform/ethanol furnished N-4-benzyloxyphenyl-N-2-(2,4-dinitrophenylamino)-phenyltoluene-4-sulphonamide 415 (45%) as bright yellow crystals, m.p. 151-2°. (Found: C, 62.8; H, 4.4; N, 9.2. $C_{32}H_{36}N_4O_7S$ requires C, 62.9; H, 4.3; N, 9.2%). ν_{max}^{KBr}: 3260cm^{-1} (N-H). τ($CDCl_3$): 1.84-3.33 (20H, m, aromatics); 5.1 (2H, s, PhC$\underline{H_2}$O); 5.35 (1H, s, exchangeable with D_2O, N$\underline{H}$); 7.6 (3H, s, $C_6H_4C\underline{H_3}$).

Cleavage of benzyl ethers

The N-benzoyl derivative 412 (5g, 0.009 mol) was dissolved in trifluoroacetic acid (30cm^3) and allowed to stand for 18h in a stoppered flask. Evaporation of the dark solution left a brown solid which was recrystallised from acetone/petroleum (b.p. 60-80°) to afford N-2-benzamidophenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 416 (3g, 73%) as colourless crystals, m.p. 246-8°. (Found: C, 67.9; H, 4.9; N, 6.0. $C_{26}H_{22}N_2O_4S$ requires C, 68.1; H, 4.8; N, 6.1%). ν_{max}^{KBr}: 3400 (O-H); 3180cm^{-1} (N-H). τ(d_6DMSO): 2.02-3.5 (17H, m, aromatics); 7.68 (3H, s, $C_6H_4C\underline{H_3}$).

Similar treatment of the 2,4-dinitrophenyl derivative 415 produced N-2-(2,4-dinitrophenylamino)-phenyl-N-4-hydroxyphenyl-toluene-4-sulphonamide 419 (70%) as bright yellow prisms, m.p. 188-188.5° from chloroform/ethanol. (Found: C, 58.1; H, 4.1; N, 10.5. $C_{25}H_{20}N_4O_7S$ requires C, 57.7; H, 3.9; N, 10.7%). ν_{max}^{KBr}: 3380 (OH); 3310cm^{-1} (NH). τ(d_6DMSO): 1.8-3.52 (15H, m, aromatics); 7.52 (3H, s, $C_6H_4C\underline{H_3}$).

The bis-sulphonamide 413 (3g, 0.0056 mol), ethanol (250cm^3), and concentrated hydrochloric acid (250cm^3) were heated together under reflux until all the solid had dissolved (72h). Evaporation and crystallisation of the resulting solid from acetone/petroleum (b.p. 60-80°) afforded N-4-hydroxyphenyl-N-2-(toluene-4-sulphonamido)-phenyltoluene-4-sulphonamide 417 (1.8g, 71%) as colourless crystals, m.p. 193.5-194.5°. (Found: C, 61.3; H, 4.9; N, 5.6. $C_{26}H_{24}N_2O_5S_2$ requires C, 61.4; H, 4.7; N, 5.5%). ν_{max}^{KBr}: 3430 (OH); 3260cm^{-1} (NH). τ(d_6DMSO): 2.34-3.44 (16H, m, aromatics); 7.66, 7.72 (6H, d, 2 x $C_6H_4C\underline{H_3}$).

Similar treatment of the N-benzyl derivative 414 produced N-2-benzylaminophenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 418 (76%) as colourless crystals, m.p. 125-6° from chloroform/petroleum (b.p. 60-80°). (Found: C, 70.2; H, 5.5; N, 6.4. $C_{26}H_{23}N_2O_3S$ requires C, 70.3; H, 5.4; N, 6.3%). ν_{max}^{KBr}: 3390cm^{-1} (OH, NH). τ(d_6DMSO): 2.37-3.5 (17H, m, aromatics); 5.65 (2H, s, $PhC\underline{H_2}N$); 7.58 (3H, s, $C_6H_4C\underline{H_3}$).

2-Amino-4'-benzyloxydiphenylamine 420

Reduction of 4-benzyloxy-2'-nitrodiphenylamine 369 with borohydride and palladium as described for the sulphonamide 396, produced the title amine 420 (75%) as shiny colourless plates, m.p. 109-110° from benzene/petroleum (b.p. 80-100°). (Found: C, 78.5; H, 6.5; N, 9.8. $C_{19}H_{18}N_2O$ requires C, 78.7; H, 6.2; N, 9.7%). ν_{max}^{KBr}: 3420, 3310cm^{-1} (NH_2 and NH). τ($CDCl_3$): 2.65-3.45 (13H, m, aromatics); 5.06 (2H, s, $PhC\underline{H_2}O$); 6.36 (2H, broad s, exchangeable with D_2O, $N\underline{H_2}$).

Treatment of the amine 420 with an equimolar amount of toluene-4-sulphonyl chloride in pyridine furnished 4-benzyloxy-2′-(toluene-4-sulphonamido)-diphenylamine 421 (94%) as colourless crystals, m.p. 161.5-162° from ethanol. (Found: C, 65.6; H, 5.2; N, 5.9. $C_{26}H_{24}N_2O_3S$ requires C, 65.9; H, 5.0; N, 5.9%). ν_{max}^{KBr}: 3360, 3130cm^{-1} (2 x NH). τ($CDCl_3$): 2.32-3.26 (17H, m, aromatics); 3.47 (1H, s, exchangeable with D_2O, NH); 5.01 (2H, s, $PhCH_2O$); 7.63 (3H, s, $C_6H_4CH_3$).

The above sulphonamide 421 was debenzylated by catalytic hydrogenolysis as described for compound 390 to afford 4-hydroxy-2′-(toluene-4-sulphonamido)-diphenylamine 422 (74%) as colourless prisms from ethyl acetate/petroleum (b.p. 80-100°), m.p.
(Found: C, 64.3; H, 4.9; N, 8.0. ($C_{19}H_{18}N_2O_3S$ requires C, 64.4; H, 5.1; N, 7.9%). ν_{max}^{KBr}: 3420, 3240cm^{-1} (2 x NH and OH). τ(d_6DMSO): 2.37-3.6 (12H, m, aromatics); 7.72 (3H, s, $C_6H_4CH_3$).

4-Benzyloxy-2′-nitrodiphenyl ether 423

Sodium hydride (50% dispersion in oil, 11.5g, 0.25 mol) was added slowly to a solution of 4-benzyloxyphenol (50g, 0.25 mol) in dry DMF (250cm^3). 1-Bromo-2-nitrobenzene (50g, 0.25 mol) and copper (I) bromide (36g, 0.25 mol) were then added and the mixture heated at 140° for 5h and poured into water (2dm^3). The mixture was extracted with chloroform (3 x 700cm^3) and the combined extracts were washed with sodium hydroxide solution (1M, 1dm^3 portions) until phenol ceased to be extracted, water (2 x 1dm^3), dried ($MgSO_4$), and evaporated to produce a black gum. Extraction with boiling petroleum (b.p. 80-100°, 3dm^3) and concentration of the extracts yielded a solid which was recrystallised from benzene/

petroleum (b.p. 80-100°) to afford the title ether (33g, 41%) as pale yellow crystals, m.p. 76-7° (lit[304] 77-8°).

Reduction of 423 by borohydride and palladium afforded 2-amino-4'-benzyloxydiphenyl ether 424 (91%) as colourless crystals, m.p. 148-9° from ethanol. (Found: C, 80.3; H, 6.2; N, 5.1. $C_{19}H_{17}NO_2$ requires C, 80.6; H, 6.0; N, 5.0%). ν_{max}^{KBr}: 3510, 3470cm^{-1} (NH_2). τ($CDCl_3$): 2.62-3.4 (13H, m, aromatics); 5.0 (2H, s, $PhC\underline{H_2}O$); 6.4 (2H, broad s, exchangeable with D_2O, $N\underline{H_2}$).

Treatment of 424 with toluene-4-sulphonyl chloride in pyridine afforded 4-benzyloxy-2'-(toluene-4-sulphonamido)-diphenyl ether 425 (77%) as colourless plates, m.p. 150-151° from ethanol. (Found: C, 69.9; H, 5.0; N, 2.9. $C_{26}H_{23}NO_4S$ requires C, 70.1; H, 5.2; N, 3.1%). ν_{max}^{KBr}: 3240cm^{-1} (NH). τ($CDCl_3$): 2.25-3.57 (17H, m, aromatics); 5.0 (2H, s, $PhC\underline{H_2}O$); 7.65 (3H, s, $C_6H_4C\underline{H_3}$); 8.31 (1H, broad s, exchangeable with D_2O, $N\underline{H}$).

The sulphonamide 425 was debenzylated by refluxing in ethanolic hydrochloric acid as described for compound 413, to furnish 4-hydroxy-2'-(toluene-4-sulphonamido)-diphenyl ether 426 (93%) as colourless crystals, m.p. 167-8°, from ethanol. (Found: C, 64.4; H, 5.0; N, 4.0. $C_{19}H_{17}NO_4S$ requires C, 64.2; H, 4.8; N, 3.9%). ν_{max}^{KBr}: 3430 (OH); 3260cm^{-1} (NH). τ(d_6DMSO); 2.33-3.5 (12H, m, aromatics); 7.66 (3H, s, $C_6H_4C\underline{H_3}$).

N-4-Benzyloxyphenyl-N-2-nitrophenylbenzamide 378

N-4-Benzyloxyphenylbenzamide (6.5g, 0.022 mol), 1-bromo-2-nitrobenzene (5g, 0.025 mol), anhydrous potassium carbonate (3g), and copper bronze (1g) were added to dry HMPT (40cm^3) and the mixture was heated at 160° for 5h. A still head was fitted to the flask and the HMPT removed under reduced pressure (160°, 1mm Hg). The residue was digested with water ($50cm^3$) and extracted with chloroform ($3 \times 50cm^3$). The combined extracts were filtered to remove the insoluble starting benzamide, washed with water ($2 \times 100cm^3$), dried ($MgSO_4$), and evaporated. Recrystallisation of the resulting solid from benzene/petroleum (b.p. 80-100°) with charcoal furnished yellow crystals of the benzamide (2.3g, 25%), m.p. 124-5°, mixed m.p. with 378 prepared by the Chapman route, 124-5°. The infrared spectra of the two samples of 378 were superimposable.

The benzamide 378, on reduction with borohydride and palladium furnished a dark solid which was difficult to recrystallise. A small amount (5%) of the pure product was obtained from ethanol after repeated treatments with activated charcoal; N-2-aminophenyl-N-4-benzyloxyphenylbenzamide 427 formed colourless crystals, m.p. 162-3°. (Found: C, 79.1; H, 5.7; N, 7.3; $C_{26}H_{22}N_2O_2$ requires C, 79.2; H, 5.6; N, 7.1%). ν_{max}^{KBr}: 3450, 3340 (NH_2); $1645cm^{-1}$ (C=O). τ($CDCl_3$): 2.21-3.17 (18H, m, aromatics); 4.5 (2H, broad s, exchangeable with D_2O, NH_2); 5.03 (2H, s, $PhCH_2O$).

The aminobenzamide 427 was treated with toluene-4-sulphonyl chloride in pyridine to afford N-4-benzyloxyphenyl-N-2-(toluene-4-sulphonamido)-phenylbenzamide 428 (80%) as colourless crystals, m.p. 159-160° from ethanol. (Found: C, 72.0; H, 5.4; N, 5.0. $C_{33}H_{28}N_2O_4S$ requires C, 72.2; H, 5.1; N, 5.1%). ν_{max}^{KBr}: 3240 (NH); $1643cm^{-1}$ (C=O). τ($CDCl_3$): 2.3-3.45 (22H, m, aromatics); 4.24 (1H, broad s, exchangeable with D_2O, NH); 5.02 (2H, s, $PhCH_2O$); 7.63 (3H, s, $C_6H_4CH_3$).

2-4′-Dihydroxybenzanilide 429

Phenyl salicylate (21.5g, 0.1 mol), 4-aminophenol (16g, 0.15 mol), and 1,2,4-trichlorobenzene (20cm^3) were heated together under reflux for 90 min. A distillation head was then fitted and the mixture distilled until phenol ceased to be collected. The residue was poured into ice-cold ethanol (100cm^3) and the resulting solid recrystallised from benzene (charcoal) to afford the title amide (20.5g, 90%) as colourless crystals, m.p. 165-6° (lit[305] 168-9°).

WORK DESCRIBED IN CHAPTER THREE

Section 3.2

Preparation of the Spirodienone 430

To a solution of N-2-Hydroxyphenyl-N-4-hydroxyphenyltoluene-4-sulphonamide 408 (2.4g, 0.068mol), in sulphur-free benzene ($1.5dm^3$) was added active manganese dioxide[307] (24g). The mixture was heated under reflux for 4h. using a Dean and Stark apparatus to effect the azeotropic removal of water. The mixture was filtered through celite and evaporated to leave a brown solid which on recrystallisation from chloroform/ethanol (charcoal) afforded the title dienone 430 (1.1g, 46%) as yellow prisms, m.p. 172-3° decomp. (Found: C, 64.5; H, 4.5; N, 3.8. $C_{19}H_{15}NO_4S$ requires C, 64.6; H, 4.3; N, 4.0%). ν_{max}^{KBr}: 1682, 1645, and $1613cm^{-1}$ (cyclohexa-2,5-dien-4-one). $\tau(CDCl_3)$: 2.3-3.36 (8H, m, aromatics); 3.38, 3.5, 3.8, 3.93 (4H, quartet, vinylics); 7.65 (3H, s, $C_6H_4\underline{CH_3}$).

Reduction of dienone 430

The dienone 430 (0.1g, 0.00028mol) was dissolved in absolute ethanol ($10cm^3$) and solid sodium borohydride (0.5g) added in portions (0.1g); the yellow solution became colourless. Water ($10cm^3$) was added and the mixture extracted with chloroform ($3 \times 10cm^3$). The combined extracts were washed with water ($2 \times 10cm^3$), dried (Na_2SO_4) and evaporated to leave a colourless solid which was recrystallised from ethyl acetate/petroleum (b.p. 80-100°) to afford colourless prisms (0.086g, 86%), m.p. 175-6°, mixed m.p. with the diphenolic sulphonamide 408 175-6°. The infrared spectra of the two samples of 408 were superimposable.

Reaction of 430 with a Grignard reagent

The dienone 430 (0.35g, 0.001 mol) was dissolved in a mixture of dry THF ($15cm^3$) and dry ether ($5cm^3$). The resulting solution was added dropwise under dry nitrogen to a magnetically stirred solution of methylmagnesium iodide, prepared by adding iodomethane (0.72g, 0.005mol) in dry ether ($5cm^3$) to magnesium (0.13g) in dry ether ($5cm^3$). After being stirred for 5h. the mixture was poured slowly into ice-cold hydrochloric acid (0.5M, $30cm^3$) and extracted with ether (3 x $20cm^3$). The combined extracts were washed with water (2 x $30cm^3$), dried (Na_2SO_4), and evaporated to leave a colourless gum (0.358g). This gum was dissolved in sodium hydroxide solution (0.5M, $20cm^3$) and dimethyl sulphate ($3cm^3$) was added in small portions with shaking between additions. The resulting alkaline mixture was extracted with ether (3 x $20cm^3$) and the combined extracts washed with water (2 x $20cm^3$), dried (Na_2SO_4), and evaporated to leave a clear gum (0.33g). This was applied to a preparative thick-layer plate (silica gel) which was developed with chloroform. The major band ($R_f = 0.82$) was separated and packed into a column (10 x 2cm) and eluted with methanol to afford a colourless crystalline solid, (0.24g) m.p. 134-5°. This material was identified as N-2-methoxyphenyl-N-3,4-dimethylphenyltoluene-4-sulphonamide by comparison with an authentic sample 388 prepared by the Goldberg-type synthesis, mixed m.p. 134-5°. The infrared spectra of the Grignard product and 388 were superimposable.

Section 3.3

Oxidation of the N-benzoyl derivative 416

The phenolic amide 416 (0.3g) and active manganese dioxide

(3g) were heated together in benzene (600cm^3) for 4h. Filtration and evaporation yielded a dark red solid (0.21g) which did not melt sharply and could not be recrystallised. T.l.c. (chloroform) showed it to have an R_f value of 0.74 while 416 had a value of 0.67 and using ethyl acetate the respective values were 0.77 and 0.81. High resolution mass spectrometry showed the presence of a component with a m/e value of 458.1261 (Calc for $C_{26}H_{22}N_2O_5S$ 458.1301).

Oxidation of 419

The dinitrophenyl derivative 419 (1g) and manganese dioxide (12g) were heated together in benzene (800cm^3) for 3h. Filtration and evaporation yielded an orange solid (0.65g) which was dissolved in chloroform (1cm^3) and the resulting solution applied to two thick-layer plates which were developed with chloroform/10% ethyl acetate. The major bands ($R_f = 0.75$) were separated and eluted with methanol to afford an orange solid (0.23g), m.p. 150-5° (not sharp). ν_{max}^{KBr}: 1674, 1652 and 1618 cm^{-1} (possible cyclohexa-2,5-dien-4-one absorptions).

Oxidation of the bis-sulphonamide 417

The phenolic sulphonamide 417 (0.7g, 0.0014mol) and manganese dioxide (7g) were heated together under reflux in benzene (400cm^3) for 3h. Filtration and evaporation yielded a yellow solid which was recrystallised from chloroform/ethanol to afford N,N-bis-(toluene-4-sulphonyl)-2,3-dihydro-1H-benz[d]imidazole-2-spiro-1′-cyclohexa-2′,5′-dien-4′-one 445 (0.4g, 58%) as pale yellow prisms, m.p. 233-4°. (Found: C, 61.7; H, 4.6; N, 5.3. $C_{26}H_{22}N_2O_5S_2$ requires

C, 61.7; H, 4.4; N, 5.5%). ν_{max}^{KBr}: 1682, 1643 and 1618cm^{-1} (cyclohexa-2,5-dien-4-one). τ(CDCl$_3$): 2.21-3.32 (12H, m, aromatics); 3.37, 3.53, 3.7, 3.87 (4H, quartet, vinylics); 7.62 (3H, s, $C_6H_4C\underline{H_3}$).

Reduction of 445

The dienone 445 (0.2g) was reduced with sodium borohydride as described for compound 430. A colourless crystalline solid (0.18g) was obtained, m.p. 195-6° from acetone/petroleum (b.p. 60-80°), mixed m.p. with 417, 195-6°. The infrared spectra of the product and of 417 were superimposable.

Reaction of the dienone 445 with a Grignard Reagent

A solution of the dienone 445 (0.5g, 0.001mol) in dry THF (30cm^3) was added dropwise under dry nitrogen to a magnetically stirred solution of methylmagnesium iodide, prepared by adding iodomethane (1.6g) in dry ether (15cm^3) to magnesium (0.4g) in dry ether (15cm^3). The reaction was worked up as described for the reaction of 430 with the Grignard, to yield after evaporation, a colourless solid (0.42g) which t.l.c. (chloroform) showed to contain two components. The major one had an R_f value of 0.89 and the minor one an R_f of 0.67. High resolution mass spectrometry of the crude product showed the presence of a component with an m/e value of 534.1589 (0.3% abundance) (Calc for $C_{29}H_{30}N_2O_4S_2$, 534.1647) and a component with m/e = 520.1504 (7.1% abundance) (Calc for $C_{28}H_{28}N_2O_4S_2$, 520.1491).

The white solid was dissolved in chloroform (2cm^3) and applied to a thick-layer plate which was developed with chloroform. The band corresponding to the spot with $R_f = 0.89$ was separated and eluted with methanol to afford colourless crystals m.p. 175-6°. This substance was compared with authentic 447, prepared as follows.

The nitrosulphonamide 397 (39.6g, 0.1mol) was dissolved in 1,4-dioxan (300cm^3) and palladised charcoal (3g) added. The mixture was shaken under hydrogen (50p.s.i.) until the uptake of gas ceased. Filtration and evaporation followed by recrystallisation from ethanol afforded N-2-aminophenyl-N-3.4-dimethylphenyltoluene-4-sulphonamide 446 (28g, 79%) as colourless crystals, m.p. 111-2°. (Found: C, 68.9; H, 6.1; N, 7.9. $C_{21}H_{22}N_2O_2S$ requires C, 68.9; H, 6.0; N, 7.7%). ν_{max}^{KBr}: 3435, 3352cm^{-1} (NH_2) τ($CDCl_3$): 2.32-3.46 (11H, m, aromatics); 5.79 (2H, broad s, exchangeable with D_2O, $N\underline{H}_2$); 7.59 (3H, s, $SO_2C_6H_4C\underline{H}_3$); 7.82 (6H, s, 2 x $NC_6H_3C\underline{H}_3$).

The amine 446 was treated with toluene-4-sulphonyl chloride in pyridine to furnish N-3,4-dimethylphenyl-N-2-(toluene-4-sulphonamido)-phenyltoluene-4-sulphonamide 447 (96%) as colourless crystals, m.p. 176-7° from chloroform/ethanol. (Found: C, 64.7; H, 5.4; N, 5.2. $C_{28}H_{28}N_2O_4S$ requires C, 64.6; H, 5.4; N, 5.4%). ν_{max}^{KBr}: 3320cm^{-1}(N-H). τ($CDCl_3$): 2.28 (1H, s, exchangeable with D_2O, N$\underline{H}$); 2.35-3.3 (15H, m, aromatics); 7.6, 7.7 (6H, d, 2 x $SO_2C_6H_4C\underline{H}_3$); 7.82, 7.88 (6H, d, 2 x $NC_6H_3C\underline{H}_3$).

The authentic 447 and the Grignard product had mixed m.p.

175-6° and their infrared spectra were superimposable.

Section 3.4

Oxidation of the amine 410 to 449

The aminophenol 410 (3g, 0.0085 mol) and active manganese dioxide (30g) were added to sulphur-free benzene (1dm^3) and heated under reflux for 2h. with mechanical stirring. Filtration and evaporation afforded a black solid (1.2g) which was dissolved in chloroform (5cm^3) and applied to five thick-layer plates (silica gel). The plates were developed using ethyl acetate and the bands containing the bright yellow spot (R_f = 0.78) were combined and eluted with methanol to give bright yellow plates (0.57g), m.p. 196-7° from benzene. (Found: C, 62.7; H, 7.5; N, 4.1. $C_{19}H_{14}N_2O_4S$ requires C, 62.6; H, 7.7; N, 3.9%). Found m/e = 366.0701, (calc for $C_{19}H_{14}N_2O_4S$, 366.0675). ν^{KBr}_{max}: 1678, 1630 and 1608cm^{-1} (cyclohexa-2,5-dien-4-one type absorptions). $\tau(CDCl_3)$: 1.96-2.72 (4H, quartet, aromatics); 3.18-3.8 (7H, m, vinylics); 7.54 (3H, s, $C_6H_4CH_3$).

Section 3.5

Oxidation of the diphenyl ether 426

The phenolic sulphonamide 426 (0.8g, 0.0022mol) and active manganese dioxide (8g) were added to benzene (500cm^3) and heated under reflux for 3h. Filtration and evaporation followed by recrystallisation from chloroform/ethanol yielded yellow crystals (0.39g, 49%), m.p. 171-2° decomp, mixed m.p. with 430 171-2° decomp. The infrared spectra of the two samples of 430 were superimposable.

Oxidation of the diphenylamine 422

The phenolic sulphonamide 422 (2.2g, 0.0061 mol) and active manganese dioxide (22g) were added to benzene (1.6dm^3) and heated under reflux for 3h. Filtration and evaporation gave a dark solid which was dissolved in chloroform (10cm^3) and applied to five thick-layer plates (silica gel). These were developed with chloroform and showed two yellow bands (R_f values 0.66 and 0.4). The yellow bands corresponding to the $R_f = 0.4$ component were combined and eluted with acetone to afford bright yellow plates (0.83g) m.p. 196-7° from benzene. The mixed m.p. with the oxidation product of 410 was 196-7° and the two infrared spectra were superimposable. The product of this oxidation gave an intense dark blue colour when treated with sodium hydroxide solution (0.01M).

Oxidation of the benzanilide 429

The diphenolic benzanilide 429 (1.1g, 0.005 mol) and active manganese dioxide (11g) were added to benzene (1dm^3) and the mixture heated under reflux for 4h. Filtration and evaporation afforded a grey solid (0.2g) which was dissolved in chloroform (2cm^3) and applied to a thick-layer plate (silica gel). The plate was developed with chloroform and the major fast-running band ($R_f = 0.72$) was separated and eluted with acetone to give an off-white solid (0.06g), m.p. 140-5°. ν_{max}^{KBr}: 1680, 1647 and 1614cm^{-1} (possible cyclohexa-2,5-dien-4-one absorptions).

WORK DESCRIBED IN CHAPTER FOUR

Section 4.3

Growth of the fungus

Polyporus versicolor, strain 28a, was obtained as a culture growing on an agar slope from the Building Research Establishment, Princes Risborough, Aylesbury, Bucks. The organism was subcultured on to agar plates grown at 37° and stored at 4°. The liquid medium[411] was prepared as follows (all chemicals were A.R. grade where available).

glucose	20g
L-asparagine	2.5g
DL-phenylalanine	0.15g
adenine	0.0275g
thiamine hydrochloride	0.00005g
KH_2PO_4	1.0g
$Na_2HPO_4 \cdot 2H_2O$	0.1g
$MgSO_4 \cdot 7H_2O$	0.5g
$CaCl_2$	0.01g
$FeSO_4 \cdot 7H_2O$	0.01g
$MnSO_4 \cdot 4H_2O$	0.001g
$ZnSO_4 \cdot 7H_2O$	0.001g
$CuSO_4 \cdot 5H_2O$	0.002g
distilled water to $1dm^3$.	

The medium was equally distributed between five conical flasks ($250cm^3$) which were then sterilised by autoclaving (120° for 30 min). One plate of freshly grown fungus was divided into ten pieces, two of which were added to each flask under sterile conditions. The flasks were then incubated at 25° for 7 days at which point a solution of 2,5-dimethylaniline in 50% aqueous ethanol (0.2M) was added so that the final concentration of 2,5-dimethylaniline in the flasks was $4 \times 10^{-4}M$. After a further 7 days incubation at 25° the cultures were harvested. Glass beads (100g) were added to each flask which was then shaken manually for 10 min. to break up the mycelial mat. The resulting mycelial suspensions were filtered through Whatman No 1 paper and the filtrates stored at -20° in polyethylene bottles.

Assay of laccase activity[412]

Reaction mixtures contained catechol ($1x10^{-6}$mol), acetate buffer (pH = 4.0, $5x10^{-5}$mol), and enzyme solution ($0.1cm^3$) in a total volume of $3cm^3$. The mixtures were incubated at 37° for 15 min. and the absorptions at 400nm were determined against a catechol 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 min.

Preliminary experiments with laccase

Vanillin (0.2g) was dissolved in 25% aqueous ethanol ($50cm^3$) and laccase solution (activity = 3.35 units/cm^3, $25cm^3$) was added. The mixture was incubated at 37° for 24h and the resulting buff precipitate filtered and dried to afford dehydrovanillin (0.035g), m.p. 304-5° (lit[481] 305°).

2,6-Dimethoxyphenol (0.1g) on similar treatment produced dark blue needles of 3,3′,5,5′-tetramethoxydiphenoquinone (coerulignone), (0.023g), m.p. 289-290° (lit[482] 291-3°). 2,6-Di-t-butylphenol when incubated with laccase solution gave a turbid yellow solution which was extracted with chloroform ($2x100cm^3$). The combined extracts were dried ($MgSO_4$) and evaporated to leave a red gum, t.l.c. (chloroform) of which showed the presence of two spots (R_f values 0.76 and 0.67) in addition to unreacted phenol (R_f= 0.89).

These two spots were shown to be 3,3',5,5'-tetra-t-butydiphenoquinone 486 and 1,1'-bis-(3,5-di-t-butylcyclohexa-2,5-dien-4-one) 487 respectively by t.l.c. comparison with samples prepared by literature methods.[413,414]

Synthesis of isoquinoline-1-carboxylic acids and dihydroisoquinolines

3-Hydroxy-4-methoxyphenethylamine hydrochloride (8g, 0.04mol) and pyruvic acid (6g, 0.068mol) were dissolved in water (200cm^3) and the pH adjusted to 6.0 with ammonia solution. After standing at 25° for 7 days the resulting crystals were recrystallised from aqueous methanol to afford 6-hydroxy-7-methoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 490 (11.6g, 50%) as colourless crystals, m.p. 251-2° (lit[419] 254°).

The dihydroisoquinoline hydrochlorides 492 and 493 were prepared according to the literature methods.[420,421] 3-Benzyloxy-4-methoxyphenethylamine hydrochloride was heated with formic acid at 180° until the bubbling ceased. The resulting crude N-formyl derivative was heated under reflux for 90 min. with phosphorus oxychloride in toluene; petroleum (b.p. 40-60°) was then added and the resulting brown gum was treated with a small amount of methanol. Trituration with ether then afforded the crystalline dihydroisoquinoline hydrochloride. This was dissolved in ethanol containing palladised charcoal and shaken under hydrogen (50 p.s.i.) until the uptake of gas ceased. Filtration and evaporation followed by recrystallisation from methanol/ether furnished 6-hydroxy-7-methoxy-3,4-dihydroisoquinoline hydrochloride 492 (46% overall yield) as colourless crystals m.p. 205-6° (lit[421] 207-8°).

In analogous fashion was prepared (via the N-acetyl derivative), 6-hydroxy-7-methoxy-1-methyl-3,4-dihydroisoquinoline hydrochloride 493 (57% overall yield) as colourless crystals, m.p. 262-3° (lit[421] 261-3°) from ethanol.

3,4-Dimethoxy-β-phenethylamine (homoveratrylamine) was heated at 200° with formic acid to afford the N-formyl derivative as a colourless solid, m.p. 39-40° (lit[424] 40-42°). This was cyclised with phosphorus oxychloride in toluene to afford 6,7-dimethoxy-3,4-dihydroisoquinoline hydrochloride 499 as pale yellow crystals, m.p. 110-112° from methanol/ether. (Picrate, m.p. 200-201°, lit[424] 201-3°).

The hydrochloride 499 (6g, 0.03 mol) was dissolved in water ($50cm^3$) which was made basic with sodium hydroxide solution and extracted with dichloromethane ($3x20cm^3$). The combined extracts were mixed with a solution of potassium cyanide (10g) in water ($30cm^3$) and stirred magnetically while benzoyl chloride (13g) was added dropwise. After being stirred for 3h the dichloromethane layer was washed with hydrochloric acid (10%, $100cm^3$), sodium hydroxide solution (5%, $100cm^3$) water ($2x100cm^3$) and dried (Na_2SO_4). Evaporation gave a solid which was recrystallised from chloroform to furnish 2-benzoyl-1-cyano-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline 501 (4.8g, 70%), m.p. 215-6° (lit[425] 215-6°) as colourless crystals.

The Reissert compound 501 (2g, 0.0062mol) was added to phosphoric acid (85%, $15cm^3$) and heated at 120° under nitrogen for 20 min. The resulting red solution was cooled and added to ice-water (20cm^3); the precipitate of benzoic acid was filtered off and the residue adjusted to pH = 7.0 with concentrated ammonia solution and left at 4° overnight. The resulting crystalline solid was recrystallised from methanol to afford 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 497 (1g, 68%) as colourless crystals, m.p. 245-7° decomp (lit^{426} 252-5°).

The Reissert compound 501 (3.2g, 0.01mol) and a dispersion of sodium hydride in oil (0.6g, 0.012mol) were added to dry DMF (25cm^3) and stirred magnetically for 12h with the exclusion of moisture. Iodomethane (3g, 0.02mol) was added dropwise to the deep red solution of the Reissert anion and stirring was continued for a further 24h. The solution was then poured into water (200cm^3) and extracted with chloroform (2x200cm^3). The combined extracts were washed with hydrochloric acid (10%, 200cm^3), sodium hydroxide solution (5%, 200cm^3) and water (2x200cm^3), dried (Na_2SO_4) and evaporated. Recrystallisation of the resulting solid from ethyl acetate/chloroform furnished 2-benzoyl-1-cyano-6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline 502 (2.15g, 65%) as colourless crystals, m.p. 205-6°. (Found: C, 71.6; H, 5.9; N, 8.2. $C_{20}H_{20}N_2O_3$ requires C, 71.4; H, 5.9; N, 8.3%). ν_{max}^{KBr}: 2240 ($C \equiv N$); 1653cm^{-1} ($C = O$). τ($CDCl_3$): 2.5 (5H, s, phenyl aromatics); 2.95, 3.37 (2H, d, isoquinoline aromatics); 6.07; 6.11 (6H, d, 2xOC$\underline{H_3}$); 6.4, 7.15 (4H, 2 triplets, C$\underline{H_2}$-C$\underline{H_2}$); 7.89 (3H, s, 1-C$\underline{H_3}$).

The 1-methyl Reissert 502 (5g, 0.015mol) was hydrolysed by 85% phosphoric acid as described for compound 501 to afford

6,7-dimethoxy-1-methyl-1,2,3,4-tetrahydroisoquinoline-1-carboxylic acid 500 (2.22g, 59%) as colourless crystals, m.p. 298-300° decomp from aqueous acetic acid. (Found: C, 61.9; H, 6.8; N, 5.2. $C_{13}H_{15}NO_4$ requires C, 62.1; H, 6.8; N, 5.6%). ν_{max}^{KBr}: 2880, 2780, 2650, 2430 (amino acid salt); 1640 (C = 0).

3,4-Dimethoxyphenethylamine was heated with acetic acid at 200° for 90 min to afford the N-acetyl derivative as a colourless solid m.p. 89-90° (lit[424] 94-5°). This was then cyclised with phosphorus oxychloride in toluene to furnish 6,7-dimethoxy-1-methyl-3,4-dihydroisoquinoline hydrochloride as pale yellow crystals (62%) m.p. 195-6° (lit[427] 200°).

Section 4.4

The isoquinaldic acid 490 (0.5g) was dissolved in a mixture of phosphate buffer (0.1M, pH = 6.0, 200cm^3) and ethanol (50cm^3); laccase solution (activity 2.8 units/cm^3, 100cm^3) was added and the mixture incubated at 35°. Further laccase solution (60cm^3) was added after 24 and 48h, and after 72h the mixture was made basic (pH = 10) with ammonia solution. The mixture was continuously extracted with chloroform (1dm^3) for 12h and the organic layer then dried (Na_2SO_4) and evaporated to yield a pale brown gum (0.069g).

This was shown to be identical by t.l.c. (acetonitrile/15% water) with the dihydroisoquinoline 493. A picrate of the brown gum was formed which had m.p. 205-9° decomp, mixed m.p. with picrate of authentic 493, 203-7° decomp.

Reactions for spectrophotometric assay

The reaction mixture contained the isoquinaldic acid (0.05-1.0cm^3 of a 0.0005M solution), laccase solution (circa 2 units/cm^3, 0.1cm^3) and phosphate buffer (0.1M, pH = 6.0) to a final volume of 3.0cm^3. The increase in absorbance at the required wavelength was measured against a blank containing the acid and buffer but no enzyme.

Horseradish Peroxidase

The enzyme was obtained from Sigma Chemical Co. as a salt-free powder (type 1) with an RZ value of 0.57. It was assayed for activity by the method of Devlin.[428] 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 (0.01cm^3), and phosphate buffer (0.1M, pH = 6.0, 2.9cm^3). The mixture was read after 5 min at 470 nm against a blank containing guaiacol, peroxide and buffer. One unit of activity is defined as that amount of enzyme which will cause an increase in the optical density of 1.0 unit in 5 min.

Reactions for spectrophotometric assay

Reaction mixtures contained the isoquinaldic acid (0.05-1.0cm^3 of a 0.0005M solution), hydrogen peroxide solution (0.00087M, 1.0cm^3), peroxidase solution [0.1g/dm^3 in phosphate buffer (0.1M, pH = 6.0), 1.0cm^3] and phosphate buffer (0.1M, pH = 6.0) to a final vol of 3.0cm^3. The increase in absorbance at the required wavelength was measured against a blank containing the acid, peroxide and buffer.

WORK DESCRIBED IN CHAPTER FIVE

Section 5.3

a) Synthesis of the N-formyl compound 520 via 7-O-benzylcoclaurine

4-Benzyloxy-3-methoxy-β-phenethylamine (10g, 0.04mol) and 4-hydroxyphenylacetic acid (6g, 0.04mol) were heated together at 190° for 2h under nitrogen. Recrystallisation of the resulting solid from benzene afforded N-(4-benzyloxy-3-methoxyphenethyl)-4-hydroxyphenylacetamide[453] 515 as colourless crystals m.p. 148-50°.

The amide 515 (6g, 0.015mol) was dissolved in dry acetonitrile ($120cm^3$) containing phosphorus oxychloride (18g) and heated under reflux for 1h. The solvent was removed under reduced pressure and methanol ($10cm^3$) followed by ether ($200cm^3$) was added to the residual brown gum. The resulting crystalline dihydroisoquinoline hydrochloride 516 was dissolved in ethanol ($50cm^3$) and sodium borohydride (3g) was added in portions. The mixture was warmed on the steam bath for 30min, poured into water ($200cm^3$) which was then brought to pH = 7.0 with dilute hydrochloric acid, and extracted with chloroform ($3x100cm^3$). The combined extracts were dried (Na_2SO_4) and evaporated to leave 7-O-benzylcoclaurine 517 (0.8g, 14%) as colourless crystals, m.p. 151-2° (lit[453] 153-4°) from benzene.

The tetrahydroisoquinoline 517 (0.2g) was stirred for 24h with a mixture of acetic anhydride ($10cm^3$) and formic acid ($6cm^3$). The resulting mixture was poured into sodium hydroxide solution (2M, $200cm^3$) and heated under reflux for 30min. Extraction with chloroform ($2x100cm^3$) and drying of the extracts (Na_2SO_4) followed by evaporation afforded an amorphous solid (0.16g) which was recrystallised with difficulty from ethanol to form crystals, m.p. 189-90°, mixed m.p. with authentic 520, 187-8°.

b) Synthesis of 520 via the ethoxycarbonyl derivative

The phenolic amide 515 was treated with ethyl chloroformate according to the literature method[453] to afford N-(4-benzyloxy-3-methoxyphenethyl)-4-ethoxycarbonyloxyphenylacetamide 518 (91%) as colourless crystals, m.p. 103-4° (lit[453] 105.5-106°) from benzene/petroleum (b.p. 80-100°).

The amide 518 was cyclised using phosphorus oxychloride in toluene followed by dilution with petroleum (b.p. 40-60°) and treatment of the resulting gum with methanol and then ether. Recrystallisation of the resulting solid from ethanol/ether furnished 7-benzyloxy-1-(4-ethoxycarbonyloxybenzyl)-6-methoxy-3,4-dihydroisoquinoline hydrochloride 519 as pale yellow plates, m.p. 194-5° (lit[416] 195-6°).

The dihydroisoquinoline hydrochloride 519 (4g, 0.009 mol), formamide ($75cm^3$), and formic acid ($15cm^3$) were heated together under reflux for 3h. The resulting solution was poured into water ($500cm^3$) and extracted with chloroform ($3x200cm^3$); the combined extracts were dried (Na_2SO_4) and evaporated to leave a colourless solid. Recrystallisation from ethanol afforded 7-benzyloxy-2-formyl-1-(4-hydroxybenzyl)-6-methoxy-1,2,3,4-tetrahydroisoquinoline 520 (2.8g, 80%) as colourless crystals, m.p. 192-3°. (Found: C, 77.7; H, 6.8; N, 3.7. $C_{25}H_{25}NO_4$ requires C, 77.5; H, 6.5; N, 3.6%). ν^{KBr}_{max}: 3330 (OH), $1643cm^{-1}$ (C = O). $\tau(CDCl_3)$: 1.95 (1H, s, NCHO); 2.38-3.65 (11H, m, aromatics); 5.07 (2H, s, $PhCH_2O$); 5.65 (1H, s, 1-C-H); 6.15 (3H, s, OCH_3); 6.95-7.95 (6H, m, aliphatics).

In a similar fashion from 1-(4-benzyloxybenzyl)-6-ethoxycarbonyloxy-7-methoxy-3,4-dihydroisoquinoline hydrochloride 521 was prepared 1-(4-benzyloxybenzyl)-2-formyl-7-hydroxy-6-methoxy-1,2,3,4-tetrahydroisoquinoline 522 (84%) as colourless crystals m.p. 167-8° from ethanol. (Found: C, 77.3; H, 6.4; N, 3.3. $C_{25}H_{25}NO_4$ requires C, 77.5; H, 6.5; N, 3.6%). ν_{max}^{KBr}: 3260 (OH), 1660cm^{-1} (C = O). $\tau(CDCl_3)$: 1.95 (1H, s, NCHO); 2.48-3.56 (11H, m, aromatics); 5.03 (2H, s, $PhCH_2O$); 5.68 (1H, s, 1-C-H); 6.18 (3H, s, OCH_3); 6.92-7.7 (6H, m, aliphatics).

Anodic oxidations

These were carried out using a potentiostat with the anode a piece of graphite felt (8x5cm) and the cathode a piece of platinum foil (1.5x1.5cm). A standard calomel electrode was used as the reference.

The phenolic amide 520 (2g, 0.005mol) was dissolved in a solution of sodium (0.12g, 0.005mol) in methanol (20cm^3) and the solvent removed to leave the sodium salt. This was dissolved in 50% aqueous methanol (100cm^3) and added to acetonitrile (300cm^3) containing tetraethylammonium perchlorate (6g). The potential was adjusted such that the current passing was 50mA and maintained to provide that current for 2.8h. During that time the potential (versus SCE) was increased from 0.26V to 0.29V; the temperature was maintained at 25°. At the end of the electrolysis hydrochloric acid (2M, 100cm^3) was added to the reaction mixture and the mixture evaporated to dryness. Water (200cm^3) and chloroform (400cm^3) were added and the organic layer washed with water (2x200cm^3), dried (Na_2SO_4) and evaporated to afford a gum (1.7g).

The above experiment was repeated with the current maintained at 20mA and the temperature at 4°; the potential was increased from 0.23V to 0.35V (versus SCE) during the course of the oxidation (7h). Workup as before gave a similar gum (1.2g).

Both materials showed similar t.l.c. behaviour; using acetonitrile/15% water as the solvent three spots were seen: unreacted <u>520</u>, $R_f = 0.77$; an elongated spot centred at $R_f = 0.4$; and baseline material, $R_f = 0.12$. The spots were detected using Dragendorff's reagent.[483] By two successive chromatographic separations on thick-layer plates (acetonitrile/15% water) a material (apparently a single spot by t.l.c.) was isolated. This was an amorphous solid which could be reprecipitated from acetone/petroleum (b.p. 60-80°), no sharp m.p., ν_{max}^{KBr}: 3420 (OH); 1660cm^{-1} (C = O). A molecular weight determination (ebullioscopic in chloroform) gave a value of 433.

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APPENDIX I

Velocities of Enzymic Decarboxylation of Tetrahydroisoquinoline-1-Carboxylic Acids

a laccase

Acid	Substrate Concentration $[10^{-5}M]$	Velocity $[10^{-9}mol\ min^{-1}]$ Run 1	Run 2
489	1.9	1.97	1.2
	3.8	2.46	1.8
	7.7	3.6	2.8
	1.5	6.4	5.4
	3.1	10.3	8.1
490	1.82	0.86	1.0
	3.6	1.5	2.1
	7.3	2.9	4.2
	14.8	5.2	7.7
	27.6	7.0	8.4
491	1.3	0.79	0.62
	2.7	1.32	1.5
	5.5	2.7	3.0
	11.0	5.1	5.4
	22.0	8.1	8.7
495	2.8	0.35	0.27
	5.6	0.64	0.52
	11.0	1.12	1.0
	22.0	1.68	1.7
	37.0	2.68	2.2

b horseradish peroxidase

Acid	Substrate Concentration $[10^{-5} \text{mol dm}^{-3}]$	Velocity $[10^{-9} \text{mol min}^{-1}]$ Run 1	Run 2
489	2.1	1.2	
	4.2	2.25	
	8.4	2.7	
	16.8	3.15	
	33.6	3.3	
	41.9	3.6	
490	0.9	1.2	1.85
	1.8	2.4	3.0
	3.6	4.3	4.6
	7.3	5.9	7.2
	14.6	6.7	1.0
490*	2.1	0.85	0.5
	4.2	1.5	1.3
	8.4	2.2	1.8
	16.9	3.0	3.0
	33.8	4.0	3.3
	42.2	4.8	3.7
495	1.6	0.75	1.3
	3.2	1.8	2.55
	6.4	3.9	5.1
	12.8	8.1	10.5
	25.6	15.0	16.5
	32.0	17.7	17.4

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