Medicinal Nitro Compounds

By

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Summary

Chapter I of this thesis contains a review of the biological properties of nitro compounds. Included in the discussion are those derivatives which have clinically-useful activity and those of a more experimental nature. Some metabolic transformations of nitro compounds are discussed, and the possibility that the toxicity and mutagenic or carcinogenic activity of these compounds may be initiated by reactive chemical species derived from the nitro group is discussed.

The use of infrared spectroscopy as a tool to explore features of the structure of nitro compounds is discussed in Chapter II. The asymmetric and symmetric stretching frequencies of the nitro group in a range of derivatives have been accurately recorded, and the influences of substitution, resonance effects, hydrogen-bonding, dipole moment and solvents examined. The infrared spectra of substituted o-nitrobenzamides are considerably simplified in the solution phase and it is possible to clearly identify both characteristic absorptions: in the solid phase spectra (KBr) this is not always the case. The infrared spectra of N-methyl-o-nitrobenzamide in chloroform is not modified by the incorporation of the de-oxygenating agent triethylphosphite either when the phosphite is in excess or at elevated temperatures. Under the conditions used no induced polarisation of the nitro group was detectable.

Photolysis of N-ethyl-o-nitrobenzanilide in ethanol afforded a complex mixture of products, amongst which are included azobenzene-2-carboxylic acid, 2'-hydroxyazobenzene-2-carboxylic acid and o-nitrosobenzoic acid. The same three products were identified in the photolysate of N-methyl-o-nitrobenzanilide. In contrast, no azo compounds were detected following photolysis of 1-(o-nitrobenzoyl)-1,2,3,4-tetrahydroquinoline and only o-nitrosobenzoic acid was characterised.

A mechanism to account for the formation of the afore-mentioned products is included in Chapter III.

The N-oxidation of the hypnotic drug methaqualone has been examined (Chapter IV). Methaqualone-N-oxide proved to be a very elusive compound and a satisfactory synthesis was not achieved: the main oxidation product was 2-nitrobenz-o-toluidide.

Contrary to previously published reports 2-nitrobenz-o-toluidide could not be detected as a human urinary metabolite of methaqualone.

Key Words

Nitro Compounds, Spectroscopy,

Photochemistry, Metabolism

ACKNOWLEDGEMENTS

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Grateful thanks are also extended to my husband for his encouragement and assistance.

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PART I

INTRODUCTION

Introduction

At the present time nitro compounds are widely used in medicine. The nitro group participates to a varying extent in reactions involving highly important biochemical and physiological responses. On the other hand threats to human and veterinary health are associated with the use of nitro compounds in medicine.[1]

Among the medicinal nitro compounds, are those which possess a substituent ortho to the nitro group in the aromatic system. This offers an intriguing area of research - ortho-nitro interactions - which has become prominent in the last decade.[2, 3] For example, some nitro compounds may undergo photo-induced oxidation, reduction or cyclisation reactions.[4] These processes, although initiated by light, may subsequently be modified by the presence of solvents or reactants.

Under some physiological conditions the possibility exists for biochemical transformation of nitro derivatives. It must be appreciated that these biological changes are the results of an interaction between some biological unit, and the entire molecule, not just a particular substituent in the drug. Thus the discussion of the biochemistry and pharmacology of the nitro group must be concerned with a molecular system having a variety of functional substituents, and capable of eliciting physiological and biochemical responses.[5]

PART I

CHAPTER I

BIOLOGICAL ACTIVITY

OF NITRO COMPOUNDS

Biological Activity Of Nitro Compounds

Introduction

It is not possible to discuss the precise details of the interdependence of chemical structure and biological activity of aromatic nitro compound due to the fragmentary knowledge in this field of research. However, by considering just some of the most important examples, the major outlines of metabolic reactions of these compounds can be described. Table I summarises the biological properties of some arylnitro compounds.

1.1 - Compounds Used In Medicine

Biologically active nitro compounds are mainly produced synthetically but a small number are found in nature. Among naturally occurring nitro compounds Chloramphenicol (1.1) is the most important from the medicinal point of view. It was also the first natural product recognised to possess an aromatic nitro group. Chloramphenicol was obtained in crystalline form from cultures of Streptomyces venezuelae. The chemical structure was established to be D-(L)-threo-1-p-nitrophenyl-2-dichloroacetamido-1,3-propanediol(1.1).[29] Because of the presence of two asymmetric carbon atoms in the molecule, there are four stereoisomers; of these only the D-threo isomer exhibits the full antibiotic activity. Its action is mainly bacteriostatic, arresting the growth of microorganisms and thus permitting the natural defences of the body to cope with the foreign organisms. Its effect is dramatic as it causes an almost complete halt in the synthesis of proteins by inhibiting the formation of a complex of ribonucleic acids and high molecular weight nucleoproteins which is a prerequisite for protein synthesis.[30] Resistance to the antibiotic is brought

Table 1

Biological Activity of Some Medicinal Nitro Compounds

Compounds	Other names	Summary of biological properties	References
Chloramphenicol	Chloromycetin	Broad spectrum antibiotic	6
Leukomycin-N		Broad spectrum antibiotic	7
Nitrofurazone	Furacin	Antitrypanosomal activity	8
Nitrofurantoin	Furan Nifuran	Bactericidal activity against urinary tract infections	9
Furazolidone	Tricofuran	Antibacterial and antiprotozoal action	8
Nifurtimox	Lampit	Antitrypanosomal activity (Chagas' disease)	10
Metronidazole	Flagyl	Antiprotozoal action against Trichomonas vaginalis	11
Niridazole	Ambilhar	Antishistosomal activity (particularly against S.haematobium)	12
N-(2-chloro-4-nitrophenyl)-5-chlorosalicyl-amide		Taenicide	13
Nitrazepam	Mogadon	Hypnotic; relief of insomnia caused by anxiety state	14
Azothioprine	Imuran	Antineoplastic activity; supresses immune response	15
Nefedipine	Adalat	Coronary vasodilator	16, 17
Nicoumalone	Sinthrome	Anticoagulant	18
Acinitrazole	Gynofon	Antitrichomonal agent	19
Nifuratel	Magmilor	Antibacterial and antiprotozoal action	20
Nimorazole	Nulogyl	Antiprotozoal activity	21
Tinidazole	Fasigyn	" "	22
Furaltadone		Antibacterial agent in veterinary use	23

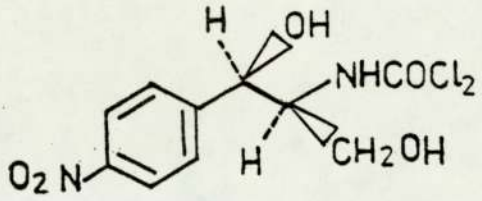
Cont. Table 1

Nifenalol		Beta-adrenergic blocking agent	24
1-Nitro-9-(4-dimethylamino-butylacridine		Antitumour activity	25
Ledakrin	Nitracrine	Antitumour activity	26
4-Nitroquinoline -1-oxide		Carcinogen	27
Ester of phosphoric acid:			
a) Parathion			
b) Paraoxon			
c) Methylparthion		Acetylcholinestrase inhibitors	28
d) Chlorthion			
e) Dicapthon			
f) Metathion			

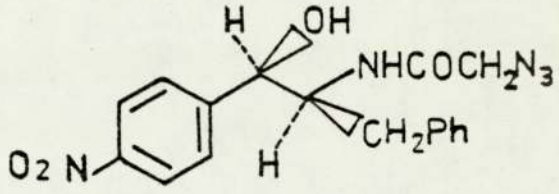
about by changes in the permeability of the bacterial cell wall to the drug, but also some organisms can degrade the drug to inactive by-products. Chloramphenicol is readily absorbed when given orally and is widely distributed in the body. It is excreted in the bile and urine. Six hours after an oral dose, about 10% is excreted unchanged in the urine; the remainder is inactivated in the liver either by conjugation with glucuronic acid or by reduction to the corresponding arylamine.[31] The liability of chloramphenicol to provoke life-threatening toxic effects, particularly bone marrow aplasia, severely limits its clinical usefulness. Apart from topical use in eye and ear infections it should never be used for minor complaints, but reserved for major outbreaks of typhoid fever. The azidoacetamido derivative has been marketed as the antibiotic Leukomycin-N(1.2). Certain compounds bearing structural similarity to Chloramphenicol (e.g. 1.3) have been found to possess antiviral activity.[32]

The antibiotic Azomycin or 2-nitroimidazole (1.4) was isolated from Nocardia mesentrica. It has a relatively low toxicity and exhibits a broad spectrum antibiotic activity even against protozoa.[33]

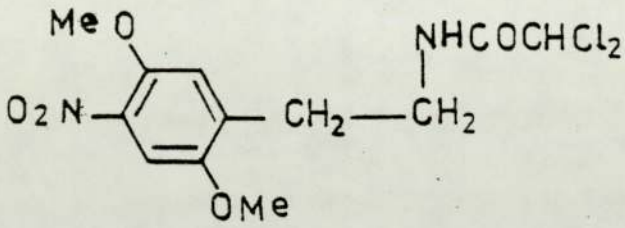
Much work[8,9] has been carried out on the structure-activity relationships of nitrofuran derivatives, which constitute an important class of antibacterial drugs.[8] It has been found that the presence of a nitro group in the 5-position of the furan ring enhances antibacterial activity. Extensions of such investigations have led to compounds such as Nitrofurazone (1.5), Nitrofurantoin (1.6) and Furazolidone (1.7). The exact role of the nitro group in these compounds is controversial: it may activate the ring carbon atom



(1.1) Chloramphenicol



(1.2) Leukomycin-N



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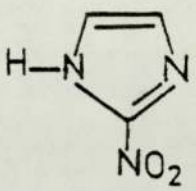
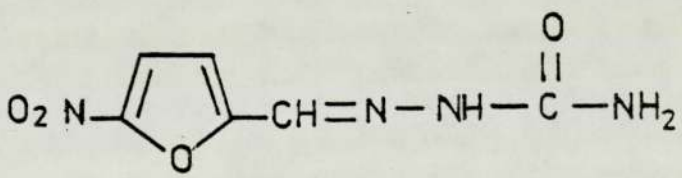
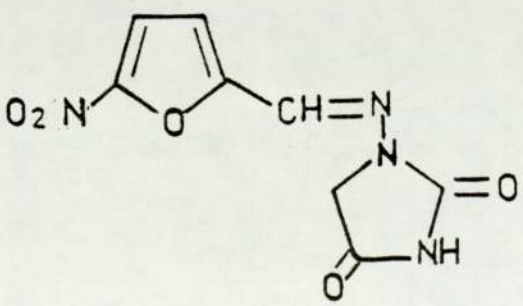


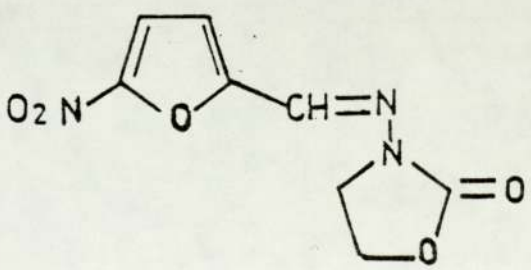
(1.4) Azomycin



(1.5) Nitrofurazone



(1.6) Nitrofurantoin



(1.7) Furazolidone

at the 5-position to attack by nucleophilic groups in biologically significant macromolecules. It has been proposed[34] that the nitrofurans exert their effect by interfering with enzymatic processes essential to bacterial growth. Nitrofurazone has been used in man as an antitrypanosomal agent; in addition, it is used in veterinary medicine for the prophylaxis of coccidiosis cases in poultry. It has been reported that Nitrofurantoin is active in urinary tract infections; it is also active against Shigella infections.[9] Nitrofurantoin is readily absorbed from the gastrointestinal tract and part is bound in blood proteins. About 40% of the dose of administered Nitrofurantoin appears unchanged in the urine.[35] Furazolidone has antiprotozoal action in addition to its antibacterial effects. Most of an administered dose is metabolised and inactivated in the intestine; about 5% is excreted in the urine together with coloured metabolites.[36] Nifuratel[20], N-(5-nitro-2-furfurylidene)-3-amino-5-methyl-mercaptomethyl-2-oxazolidinone, has also been used as an antibacterial and antiprotozoal agent. It is effective against some fungi including the notorious Candida albicans. As a therapeutic agent, it is used in the treatment of Trichomonas vaginalis infections. A metabolite, with inhibitory activity against bacteria, is excreted in the urine.[20]

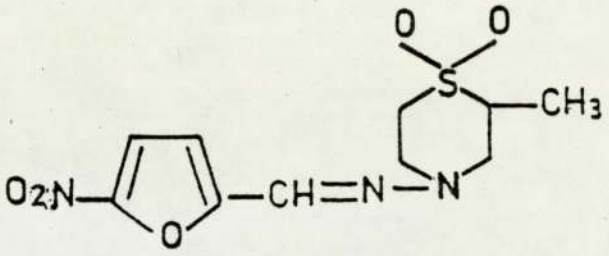
In 1972 another nitrofuran derivative was marketed as Nifurtimox (1.8). It has been used successfully for the treatment of Chagas' disease caused by Trypanosoma cruzi. It is apparently free from side effects.

Nitroimidazoles have been found to be effective against Trichomonas vaginalis which causes parasitic infections of the

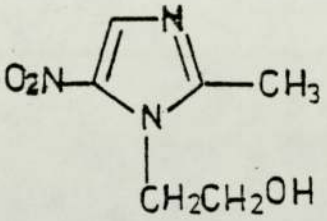
genito-urinary tract.[11] The drug Metronidazole (1.9) may be applied topically or taken orally. When administered orally, Metronidazole is absorbed well from the gastro-intestinal tract. About 50% of the dose is excreted in the urine as unchanged Metronidazole and its metabolites, including an acidic oxidation product and a glucuronide.[37] A possible mechanism for the action of Metronidazole on Trichomonos vaginalis has been suggested.[38] Unchanged drug penetrates the cell where the nitro group is reduced to a reactive intermediate (possibly a hydroxylamine) which reacts with DNA and stops nucleic acid synthesis. The drug is well tolerated at therapeutic doses, but higher doses cause serious side effects, such as disorders of the central nervous system and dermal and haematological changes. Also it has been shown that Metronidazole can induce lung tumours and malignant lymphomas in mice on prolonged dosage.[39] Nimorazole[21] and Tinidazole[22] (1.10) are also 5-nitroimidazoles and have been shown to have similar activity to Metronidazole.

Recently, interest has increased in the activity of nitroimidazoles on other anaerobic bacteria important in human and veterinary medicine.

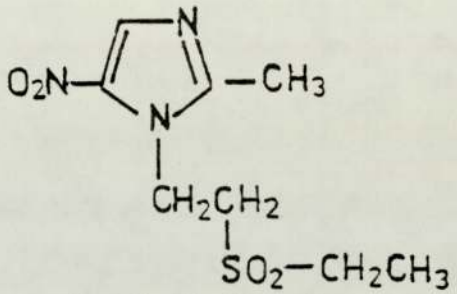
Many nitro substituted heterocyclic compounds are effective against the parasitic worm Shistosoma haematobium. The most prominent member of this group is Niridazole[12] (1.11), which contains a 5-nitrothiazolyl ring. The structure-activity relationships of a number of these compounds have been investigated.[40] A 5-nitrothiazolyl or 5-nitrofuryl ring is essential for activity together with a rigid side chain. The common mode of action of these compounds would appear to be involved with a reduction in the phosphorylase phosphatase activity of the parasitic cells.



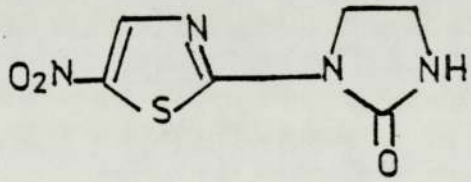
(1.8) Nifurtimox



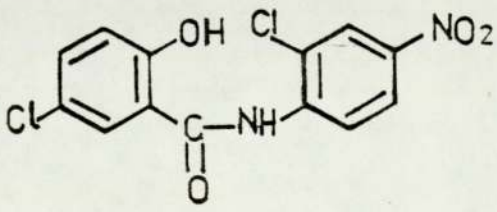
(1.9) Metronidazole



(1.10) Tinidazole



(1.11) Niridazole



(1.12)

Niridazole is slowly absorbed from the gastrointestinal tract but rapidly metabolised in the liver. Niridazole and its metabolites are widely distributed in the body tissues and are bound to plasma proteins. Dark brown metabolites are excreted in the urine and bile. In addition to its effectiveness against Schistosoma haematobium, Niridazole has been evaluated against Salmonella typhi[41] as a substitute for Chloramphenicol.

N-(2-Chloro-4-nitrophenyl)-5-chlorosalicylamide (1.12) has been introduced into clinical medicine for the treatment of tape worms.[13] The position of the nitro group is of considerable importance: also replacement of nitro by an amino group has a dyschemotherapeutic effect.

The sedative Nitrazepam (1.13) is a reliable drug largely free from side effects.[14] Overdosage does not usually produce serious side effects. It is highly suitable for psychiatric purposes and has also been used in the treatment of epilepsy. It is detoxified by reduction to the corresponding amine; also the N-acetyl derivative is found as a urinary metabolite. The derivative with a methyl group (1.14) in position 1 has been found to be one of the most potent anti-anxiety and sleep-inducing agents in clinical trials.[42]

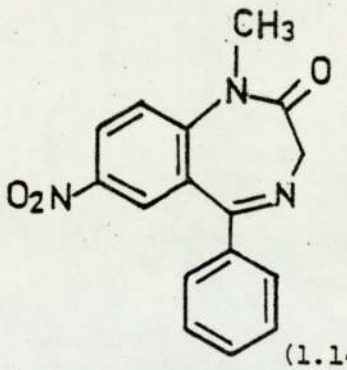
Antineoplastic activity is exhibited by a number of acridine derivatives. For example compound (1.15) exhibits activity against Ehrlich ascites sarcoma.[25] The most active derivatives in the anti-tumour nitroacridine series are those bearing the nitro group in the 1 position. The therapeutic utility of nitroacridine has been already explored.[43] The biochemical mode of action of nitroacridines such as (1.15) remains uncertain although it is reasonable to assume that the nitro compounds act by the same

mechanism as in a variety of other acridines.[25] Acridine derivatives have been shown to interfere with the replication of DNA and hence cell division. The molecular basis for this inhibition appears to depend on the specific interaction of the planar acridine ring-system with the nucleic acid helices. The acridine ring may stack between the planar stacked purine and pyrimidine bases (intercalation) and assume an arrangement stabilized by hydrophobic or charge-transfer interactions. If this is the case, then the nitro compound itself may be responsible for the cytostatic activity. Recently, Ledakrin[26] (1.16), a new antitumour nitroacridine derivative has been successfully used in medicine. The drug inhibits the biosynthesis of nucleic acids. It has been established that the active form of the drug is a metabolite, probably the hydroxylamino derivative. In vivo the drug is bound by covalent bonds to DNA and forms cross-link with complementary strands of DNA.

Azothioprine[15] (1.17) is also used as an antineoplastic agent but it is less effective. It is mainly used as an immunosuppressive drug in transplantology. The drug is slowly metabolised to 6-mercaptopurine and up to 10% is excreted unchanged in the urine.[15]

A number of N-substituted nitrobenzamides (1.18) have been found to exert CNS-depressant anticonvulsant and hypotensive activity.[44-46] The most active compounds have p-nitro groups and a small alkyl fragment attached to the amide nitrogen (1.19).

Aromatic nitro compounds exhibit a number of other interesting activities. Compound (1.20) which is marketed under the name Nefedipine was introduced into therapy as a cardiovascular dilator and antianginal agent.[16, 17] It has been evaluated in patients suffering from angina and cardiac arrythmias. In complete and perplexing contrast compound (1.21) is a powerful sweetening agent.



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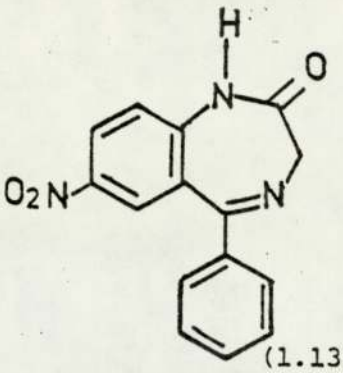
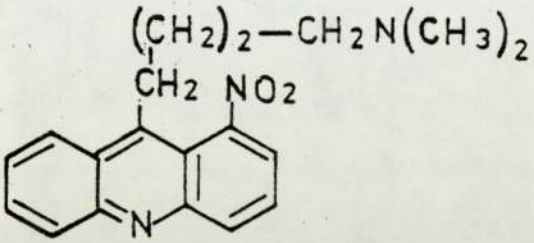


(1.13) Nitrazepam



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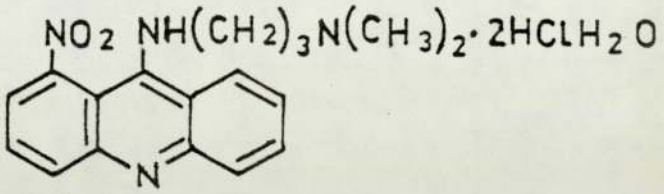
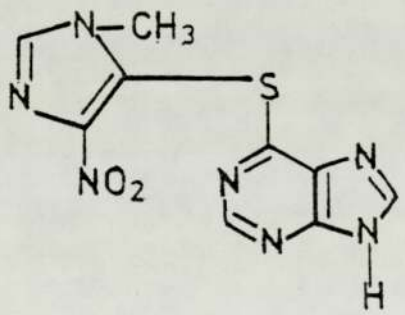
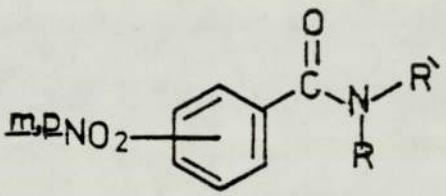


(1.16) Ledakrin

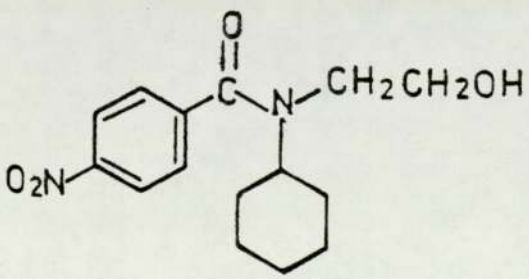


(1.17) Azathioprine



(1.18)

R = cyclohexyl's
R = alkyl, aryl or aralkyl



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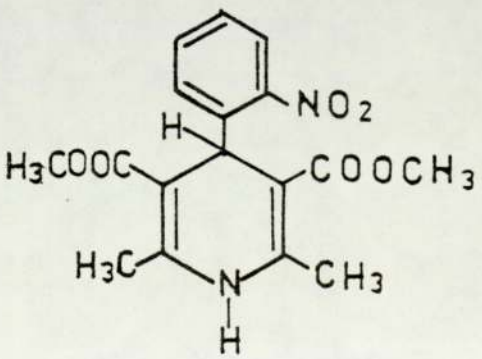


(1.20) Nefedipine

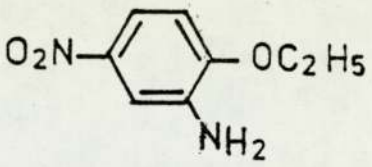
In addition to the foregoing examples it is possible to cite a variety of other nitro derivatives which exhibit a range of other biological activities. In general, these following examples have not been clinically evaluated: their purported activity is limited to experimental systems.

1.2 - Some New Arylnitro Compounds With Experimental Activity

Recently Novinson et al[47] have reported the preparation of a novel group of nitrofurfural hydrazones (1.22). According to their experiments, the compounds have antitrypanosomal activity especially against the Brazilian strain of Trypanosoma cruzi.

As part of a programme for the discovery of new antiprotozoal agents, Verge and Roffey[48] investigated the synthesis and in vivo antitrypanosomal activity of some 2-substituted thiazoles bearing a nitro-substituted thiophen ring (1.23). Some of these compounds were found to be active in curing mice and rats infected with Trypanosoma cruzi and Trypanosoma rhodesiense and moreover to have low acute toxicity. Recently the same author[49] reported another series of antiprotozoal thiazoles which have been tested in mice against the same parasite.

A number of hydrazone derivatives of 2-(4-formylstyryl)-5-nitro-1-vinylimidazole (1.24) have been synthesised and examined for antitrypanosomal properties. These compounds are clearly analogues of metronidazole (1.9). Derivatives derived from aminoguanidine, pyridylacetohydrazide chloride and dimethylamino-acetohydrazide displayed good activity when tested against Trypanosoma rhodesiense in mice.[50] Some other 5-vinyl-substituted 2-nitroimidazoles having general formula (1.25) have been reported[51] to exhibit significant antibacterial and antitrichomonal activity



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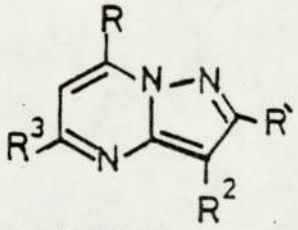

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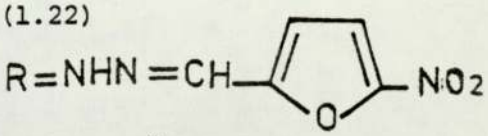
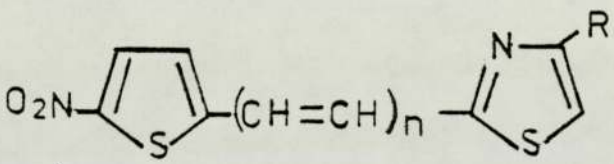
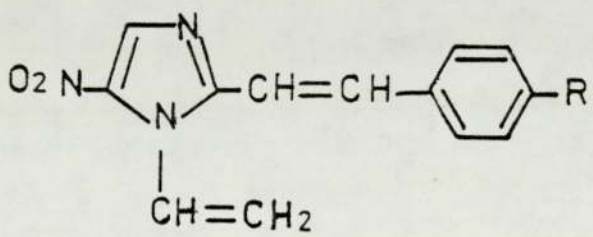


R = H's R^2 = H, aryl, CO_2Et, Cl, or Br; R^3 = H, Me or Cl



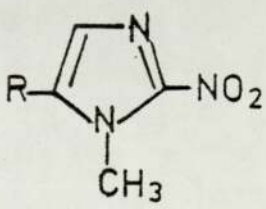
(1.23)

n = 0 or 1



(1.24)

R = -CH=NNHC(=NH) NH_2 or -CH = $NNHCOCH_2N(CH_3)_2$



(1.25)

R = substituted vinyl

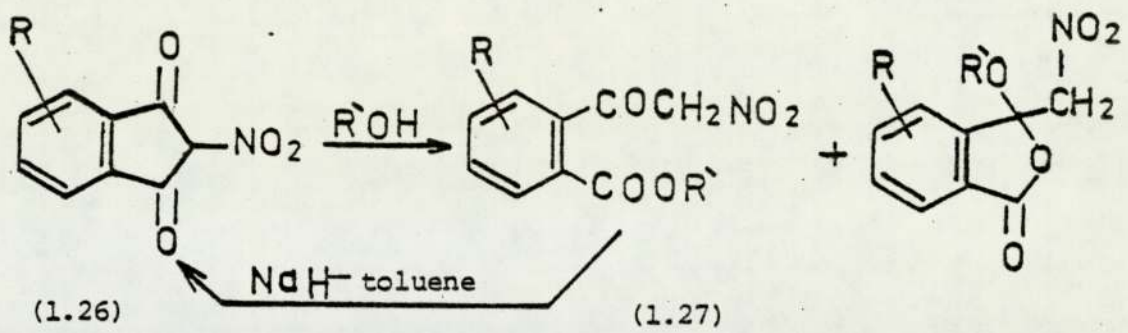
when tested in mice.

2-Nitroindan-1,3-diones (1.26) have been shown to have good antiallergic activity[52] when measured by the rat passive cutaneous anaphylaxis test. ω-Nitroacetophenones (1.27) also have antiallergic activity.[53] The latter series (1.27) can be prepared by alcoholysis of the corresponding indandiones. In addition the ω-nitroacetophenones (1.27) can be recyclised to the parent indandiones by base. The *in vivo* conversion of (1.27) to its parent indandione (scheme 2), which has been detected in rat serum, led to suggestions that this is an important factor for biological activity.[53]

1.3 - Compounds Reported to Have Toxic Activity

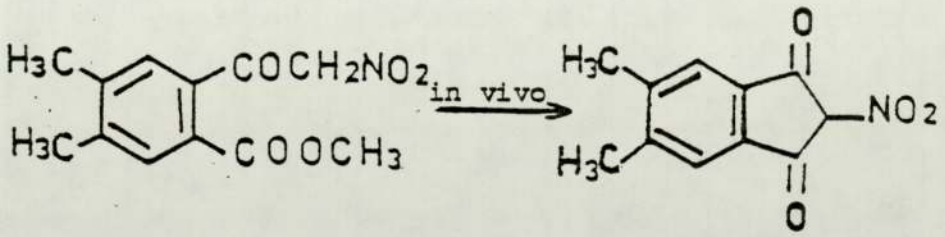
4-Nitroquinoline-N-oxide (1.28) is a potent carcinogen:[54] it also has antimicrobial activity.[55] Paul *et al*[54] proposed a model for the interaction of (1.28) and DNA on the basis of molecular orbital calculations. They postulated the formation of a complex (1.29) between the nitro compound and deoxyguanosine residues of DNA. Two sites of interaction are believed to occur: a charge transfer attraction between the nitrogen atom of the nitro group and the oxygen atom of the deoxyribose ring; and a hydrogen bond between the oxygen of the 1-oxide group and a hydrogen atom of the amino group of guanosine. The above mechanism does not preclude the possibility of some kind of covalent irreversible binding of these compounds *in vivo* with nucleophilic groups in biological macromolecules. Other workers[56] have found that a close relationship exists between the electrophilic reactivity of the carbon bearing the nitro group and carcinogenic activity.

Alkyl 2,4-dinitrophenyldisulphides undergo reaction with the sulphydryl group of the amino acid cysteine to yield

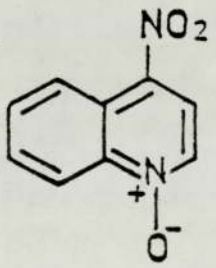


(1.26) (1.27)

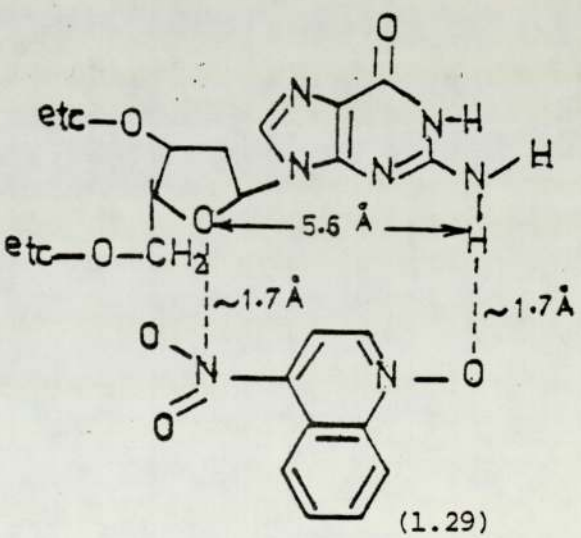
(Scheme 1)



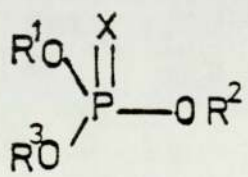
(1.27) (Scheme 2) (1.26)



(1.28)



(1.29)

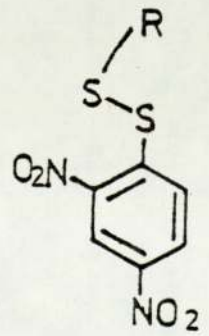


(1.31)

X = S or O

R^1 and R^2 = small alkyl group

R^3 = 4-nitrophenyl or halogenated 4-nitrophenyl



(1.30)

R = CH_2CH_2OH

2,4-dinitrothiophenols and the corresponding S-alkyl cysteine. Compounds such as the S-hydroxyethyl derivative (1.30) are potent poisons of muscular action presumably as a result of alkylation of the sulphydryl groups in the contractile proteins.[57]

1.4 - Compounds Utilized For Their Toxic Properties

Esters of phosphoric acid (1.31) are usually used as insecticides. They have irreversible anticholinesterase activity. Although toxic nitrophenols are produced on hydrolysis of the esters, poisoning occurs as the result of an accumulation of abnormally large quantities of acetylcholine at the nerve endings of the susceptible insects.[28]

1.5 - The Role Of The Nitro Group In Drugs[58]

As has been shown previously, a variety of drugs containing nitro groups are of therapeutic importance in the treatment of infectious diseases. The utility of the drug is not, in general, due to its effect on the host organism, but rather is due to the fact that the drug exerts a selective effect on the invading parasite. The toxicity of the drug is a relative property; there may be a fairly general toxicity towards a metabolic process common to both parasite and host. The decisive factors which affect the therapeutic utility of a drug are its chemical and physical properties which may be apparently unrelated to the presence of the nitro group. Another factor in affecting therapeutic use is the balance between medicinal action and detoxification of drugs bearing nitro substituents.[58] If, for example, this reduction were carried out by the microbial flora of the intestine, then it should be possible to enhance the effect of certain nitro-

substituted drugs by the simultaneous administration of drugs which diminish or abolish the activity of the intestinal flora.

Because of the frequently observed correlation between therapeutic effect and activity in the uncoupling of oxidative phosphorylation, it may be concluded that many nitro-substituted drugs act by causing severe disorder in the fundamental metabolic processes of the target organism. Because oxidative phosphorylation occurs in a very nearly identical fashion in all higher organisms, it is clear that drug toxicity toward the host, as well as the parasite, is a real possibility. Thus the therapeutic utility of a drug is highly dependent on the phenomena associated with selective permeability and drug absorption.

1.6 - Cytotoxic And Mutagenic Activity Of Some Medicinally Used Nitro Compounds

Although man has used nitrofuran derivatives as antibacterial agents, as additives to livestock food, and until recently as additives to human food, strong mutagenic and carcinogenic activity has been revealed for several derivatives. The use of nitrofurans as food preservatives is being increasingly questioned.[59] Studies on mutation induction in bacteria by many nitrofurans having different chemical structures indicated that the nitro group at the 5-position on the furan ring is essential for mutagenic activity. Mutant bacterial strains resistant to the chemicals with regard to mutation induction have been isolated, but the identity of the ultimate mutagenic agent has not been established. These mutants are assumed to be mutagenically resistant, because they have a lowered capacity to reduce the nitro group. Hence, a highly reactive reduction product intermediate seems to be

involved and to play an important role in the induction of mutation. Mammalian cells seem to have similar reduction capacities. It is possible that nitroso or hydroxylamine species are involved in cellular damage. On the other hand it has been reported that the DNA of cultured cells is damaged when cells are incubated with nitrofurazone under nitrogen.[61]

2-Amino-4-(5-nitro-2-furyl) thiazole and its N-acetylated derivatives have been reported to have carcinogenic activity in various animal species.[62]

Metronidazole is most commonly prescribed for the treatment of trichomonal infections of the urinogenital tract as has been mentioned before. It has also been suggested as an antialcohol drug, and several reports have been presented on this possible use.[63] In addition, this drug has recently been under investigation for use in treating anaerobic bacterial infections [63] and as a radiation sensitizer.[64] The carcinogenic and mutagenic properties of this widely-used drug have been the subject of several recent reports. After administration to mice, the compound was found to induce a variety of neoplasms, especially lung tumours.[63] Metronidazole was shown to be mutagenic when tested against three different species of bacteria.[63] Biochemically it has been found that reduction of Metronidazole either by bacterial nitroreductase or by rat liver preparations under anaerobic conditions is necessary for the conversion of the compound to its mutagenic form.[65] Legalar et al showed that after treatment with therapeutic doses of Metronidazole, mutagenic activity could be demonstrated in the urine of human patients.[66] (This test used Salmonella typhimurium - "The Ames Test" - as the indicator organism.)

Metronidazole produces at least two mutagenic metabolites.[63] Also it has been found that other 5-nitroimidazoles (e.g. Tinidazole), have mutagenic activity.[67]

1.7 - Metabolic Transformation Of Aromatic Nitro Compounds

In the previous survey of biologically active aromatic nitro compounds, there have been a number of examples of metabolic transformations. It will be useful to mention briefly the metabolic transformations undergone of some aromatic nitro compounds.

The metabolism of nitrobenzene in the rabbit has been investigated in some detail.[68, 69] As might be anticipated, reduction to the amino group represented an important metabolic route, but the amount of aniline found in the urine accounts for less than 1% of the administered dose. Rather p-aminophenol and its conjugates represent over 30% of the original dose isolated and identified over a 5-days period. The conjugates were largely the glucuronic acid derivatives or the N-acetyl derivative of the glucuronides. In addition conjugates of m- and p-nitrophenol were isolated in significant amounts from the urine. The enzymatic basis for formation of glucuronic acid conjugates of p-nitrophenol as a means of detoxification has been studied.[70, 71] A significant amount of the administered dose of nitrobenzene remained in the tissues for extended periods, presumably in the form of various partially metabolized derivatives. The metabolism of m-dinitrobenzene by rabbits also involves reduction and formation of aminophenols (or conjugates). m-Nitroaniline and m-phenylenediamine represented about one third of the administered dose, whilst 2-amino-4-nitrophenol and 2,4-diaminophenol isolated from the urine, together represented as much as 50% of the

administered dose. In addition 2-nitro-4-aminophenol was detected. The metabolism of nitrotoluenes by higher animals follows an interesting course in that the methyl group may be oxidized. For example p-nitrotoluene was found to be metabolised to p-nitrobenzyl alcohol and p-nitrobenzoic acid.[72] The alcohol was isolated as the glucuronic acid conjugate.

PART II

DISCUSSION OF

EXPERIMENTAL RESULTS

CHAPTER II

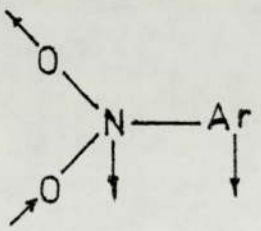
INFRARED SPECTROSCOPY OF ARYLNITRO COMPOUNDS

Infrared Spectroscopy of Aryl Nitro Compounds

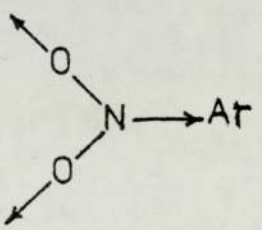
2.1 Introduction

Infrared spectroscopy of aromatic nitro compounds has been thoroughly investigated in the last few decades. Besides the application of the spectral method for the identification and characterisation of the nitro group, large numbers of papers deal with effects of other functional groups, and solvents, on the absorption frequencies of the nitro group. In particular, the effect of hydrogen bonding groups on the nitro absorptions has been evaluated.

In 1948 the standard tables of characteristic frequencies indicated that the infrared absorptions of nitro groups in general lie in the ranges 1500-1600 cm^{-1} and 1300-1350 cm^{-1};[73,74] the higher frequency absorption was attributed to the asymmetric (νasym), the lower to the symmetric stretching mode (νsym).[75]



Asymmetric
(1500-1600 cm^{-1})



Symmetric
(1300-1350 cm^{-1})

Brown[76] showed that the νasym is primarily determined by the nitrogen-oxygen stretching force constant.

Wepster[77] observed that νasym for a series of alkylnitrobenzenes is virtually constant and the corresponding frequency of the symmetrical stretching vibration is found to vary rather widely. He reported that the deviation is greater in the presence of: a) a bulky group ortho to the nitro group; b) two alkyl groups in ortho

positions with regard to the nitro group. Explanation of b) was given according to the modified valency deflection hypothesis[78-80] by the same author. An example of these variations is shown in Table 2.

Table 2

νasym and νsym of Nitro Group (cm^{-1})
of Alkylnitrobenzenes in Cyclohexane

Compounds	νasym	νsym
nitrobenzene	1526	1351
2-methylnitrobenzene	1531	1349
2,6-dimethylnitrobenzene	1537	1369

This steric effect was also noted by other workers.[81-83]

In aromatic compounds containing two nitro groups multiple frequencies are observed.[84,85] This splitting occurs in situations where one nitro group remains coplanar whilst the other is twisted out of the plane of the ring under the influence of a steric effect. This reduces the degree of aromatic conjugation and a new higher frequency band appears.

In meta and para substituted nitrobenzenes, both stretching frequencies have been correlated with structure-related parameters[86,87] such as molecular dipole moment and Hammett constants.[88] (It has been claimed that there is not a good linear relationship between νasym of the nitro group and Hammett's Constant (σ) in some meta- and para- substituted nitrobenzenes.[86]) Brown, however, showed a good linear relationship between (σ) and νasym of the nitro group; also, he reported a decrease of νasym with the presence of substituents having a more negative σ value.

Exner and co-workers[89] correlated σ with both stretching nitro group vibrations using a large number of meta- and para-

substituted nitrobenzenes. They confirmed that the asymmetric band of the nitro group is more sensitive to substitution than the symmetric mode. A plot of νasym versus σ shows scattering from the overall line, especially between the meta substituents. Their data revealed a new effect - the "meta effect". Partial linear correlation with νsym is reported in agreement with earlier workers.[76, 89, 90] The influence of solvents on the frequency of nitro group vibrations has been studied and found to be small in the absence of hydrogen bonding effects.[91]

2.2 Results and Discussion

Infrared spectra of a number of substituted nitrobenzenes have been recorded in different solvents (for details of experimental method see Chapter 5). Solvent effects on stretching frequencies of the nitro group and the correlation of substituent constants and dipole moment with these frequencies has been studied. In all linear relationships the line of best fit was calculated by regression analysis.

Frequencies of both nitro bands for several substituted nitrobenzenes in the form of potassium bromide discs or chloroform and carbon tetrachloride solutions are given in (Tables 3, 4). The precise frequencies of both bands appear in the nitrobenzene series to be related to the nature of the substituent and its position with respect to the nitro group. Of the two nitro group bands, the symmetric mode seems the more reliable in diagnosis since the presence of some substituents in the benzene ring may greatly weaken or even shift the asymmetric absorption.

Table 3

νasym of the Nitro Group in KBr, $CHCl_3$ and CCl_4 (cm^{-1})

Compounds	νasym		
	KBr	$CHCl_3$	CCl_4
nitrobenzene	1524	1526	1524
o-dinitrobenzene	1523	1545	-
m-dinitrobenzene	1537*	1538	1540
p-dinitrobenzene	1545	1553	1553
o-nitrobenzoic acid	1527	1534	-
m-nitrobenzoic acid	1531	1533	-
p-nitrobenzoic acid	1538	1527	-
m-nitrobenzaldehyde	1538	1543	-
p-nitrobenzaldehyde	1536	-	-
o-nitrotoluene	-	1523	1526
m-nitrotoluene	-	1527	1528
p-nitrotoluene	-	1515	1521
m-bromonitrobenzene	1533	1529	1534
p-bromonitrobenzene	1530	1522	1527
o-chloronitrobenzene	1527	1531	1533
m-chloronitrobenzene	1530	1536	-
p-chloronitrobenzene	1526	1521	1523
o-nitroaniline	1504	1511	1512
m-nitroaniline	1521*	1527	-
p-nitroaniline	1504	1504	-
o-nitrophenol	1527	1533	1534
m-nitrophenol	1521*	1526	-
p-nitrophenol	1512	1518	1513

* In the case of broad peaks the recorded frequency is the mid-point of the absorption

Table 4

νsym of the Nitro Group in KBr, $CHCl_3$ and CCl_4 (cm^{-1})

Compounds	νsym		
	KBr	$CHCl_3$	CCl_4
nitrobenzene	1346	1351	1351
o-dinitrobenzene	1367+	1362+	-
m-dinitrobenzene	1346	1351	1345
p-dinitrobenzene	1344	1362	1362
o-nitrobenzoic acid	1365	1350	-
m-nitrobenzoic acid	1355	1350	-
p-nitrobenzoic acid	1346	1347	-
m-nitrobenzaldehyde	1356	1350	-
p-nitrobenzaldehyde	1343	-	-
o-nitrotoluene	-	1348	1349
m-nitrotoluene	-	1349	1350
p-nitrotoluene	1349	1345	1346
m-bromonitrobenzene	1347	1347	1347
p-bromonitrobenzene	1356	1356*	1353*
o-chloronitrobenzene	1351	1352	1353
m-chloronitrobenzene	1355	1350	-
p-chloronitrobenzene	1356+	1343	1341
o-nitroaniline	1345	1345	1342
m-nitroaniline	1347*	1353	-
p-nitroaniline	1338	1336	-
o-nitrophenol	1333	1331	1331
m-nitrophenol	1349*	1353	-
p-nitrophenol	1345*	1339	1342

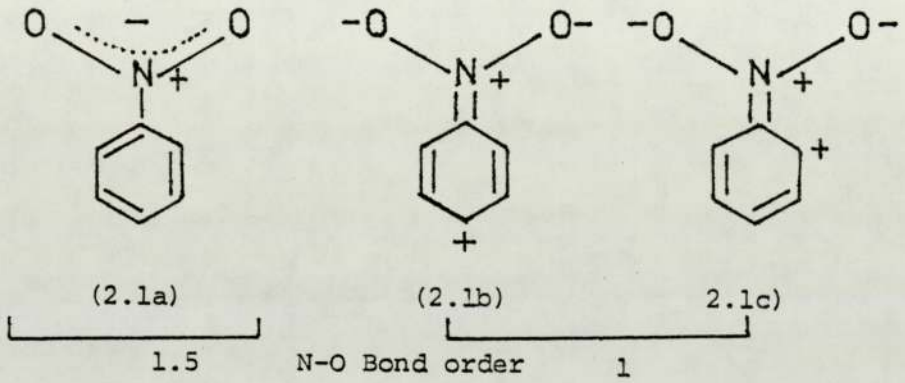
+ Split peak: the frequency recorded is the one agreed or near to the reported frequency.

* In the case of broad peaks the recorded frequency is the mid-point of the absorption.

2.3 The Asymmetric Stretching Frequency of the Nitro Group

a) Resonance effects

Brown[76] has shown that νasym is primarily determined by the nitrogen-oxygen bond order and consequently by the nitrogen-oxygen stretching force constant. Since bond orders are determined by the relative contribution of resonance forms to the overall structure in the aromatic system (2.1a ⟷ 2.1b ⟷ 2.1c), an increase in the



contribution of forms (2.1b) or 2.1c) should result in lowering of the asymmetric stretching frequencies. +M substituents (e.g. OH or NH_2) in the ortho or para positions of nitrobenzene lower the frequency of the asymmetric absorption of nitrobenzene by as much as 22 cm^{-1} in chloroform solution in the case of p-nitroaniline (Table 5). In the nitrophenols split peaks appear.

Table 5

Resonance Effect on νasym

	νasym		
	KBr	$CHCl_3$	CCl_4
nitrobenzene	1524	1526	1524
o-nitroaniline	1504	1511	1512
p-nitroaniline	1504	1504	-
o-nitrophenol	1504 1518	1511	-
p-nitrophenol	1521 1512	1518 1498	-

b) Hydrogen bonding effects

The effects of hydrogen bonding on νasym of nitro compounds even in non-polar solvents is small. For example, this absorption of o-nitrophenol (Table 3) is modified (relative to nitrobenzene) in chloroform or carbon tetrachloride by only +7 cm^{-1} and 10 cm^{-1} respectively.

c) Steric effects

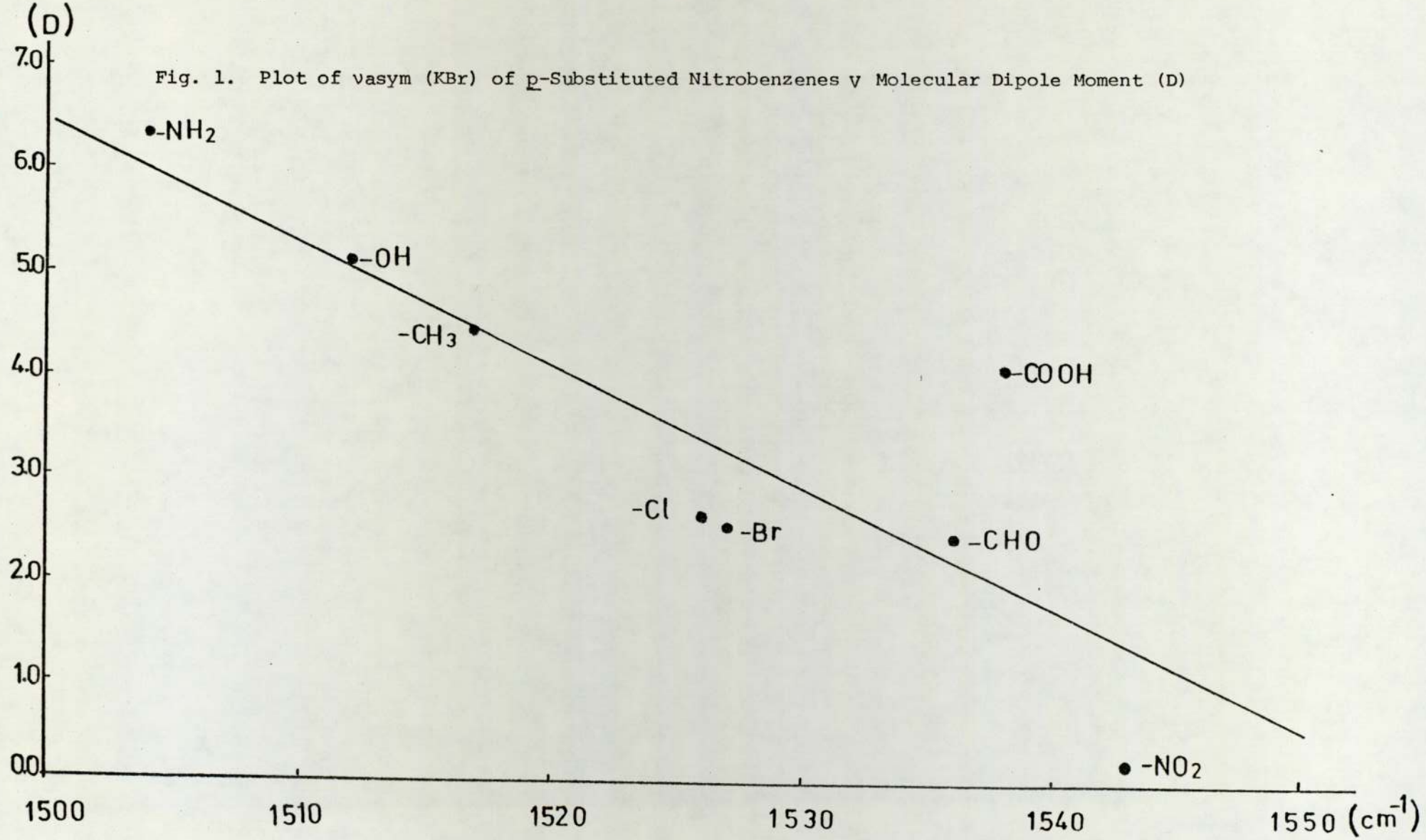
With single para-substituents the position of the asymmetric band is directly related to the electron donor or acceptor properties of the group. An extreme case is exemplified by the para-nitro group[92] in para-dinitrobenzene (νasym 1553, Table 3). Possibly one nitro group is twisted out of the plane of the ring. The last effect will, of course, reduce conjugation and explain the rise in frequency.

d) Correlation with physical constants

(i) with dipole moment (D)

A direct consequence of an increase in the contribution of more resonance forms to the overall structure is a decrease in the asymmetric stretching frequency accompanied by an increase in the dipole moment of the compound. Thus (D) and νasym in the p-substituted nitrobenzenes (Table 6) are inversely related.[89] This is confirmed when the data is plotted in graphical form (Fig. 1-3) and holds for both solid phase and solution spectra. p-Nitrobenzoic acid (Fig. 1-2) shows deviation from the overall line. However, because the carboxylic acid group creates a large dipole in opposition to that of the nitro group, the reported dipole moment of the p-nitrobenzoic acid is surprisingly high. The scatter of the other points suggests that the dipole moment for these molecules is primarily determined by the moment associated

Fig. 1. Plot of νasym (KBr) of p-Substituted Nitrobenzenes ν Molecular Dipole Moment (D)



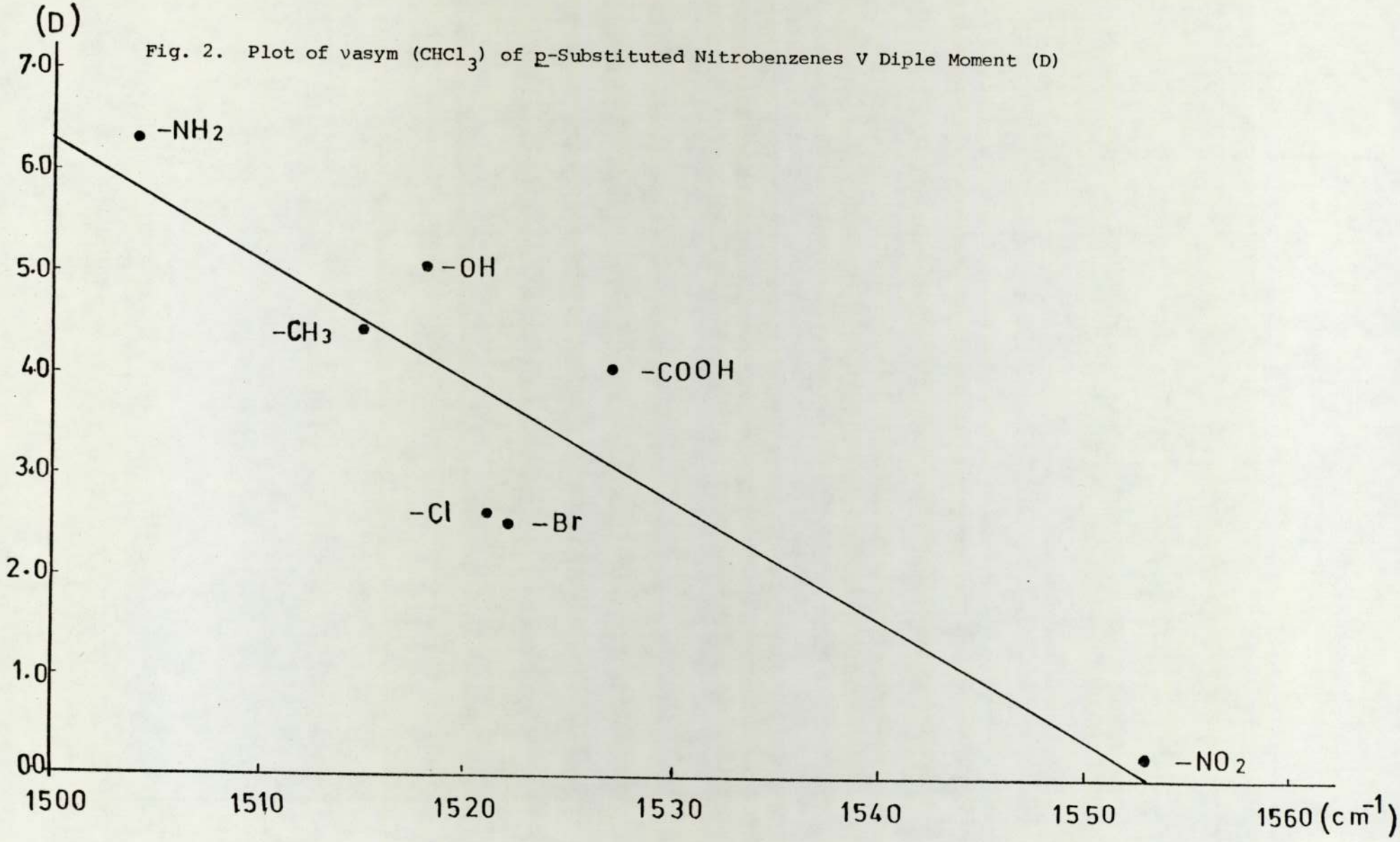
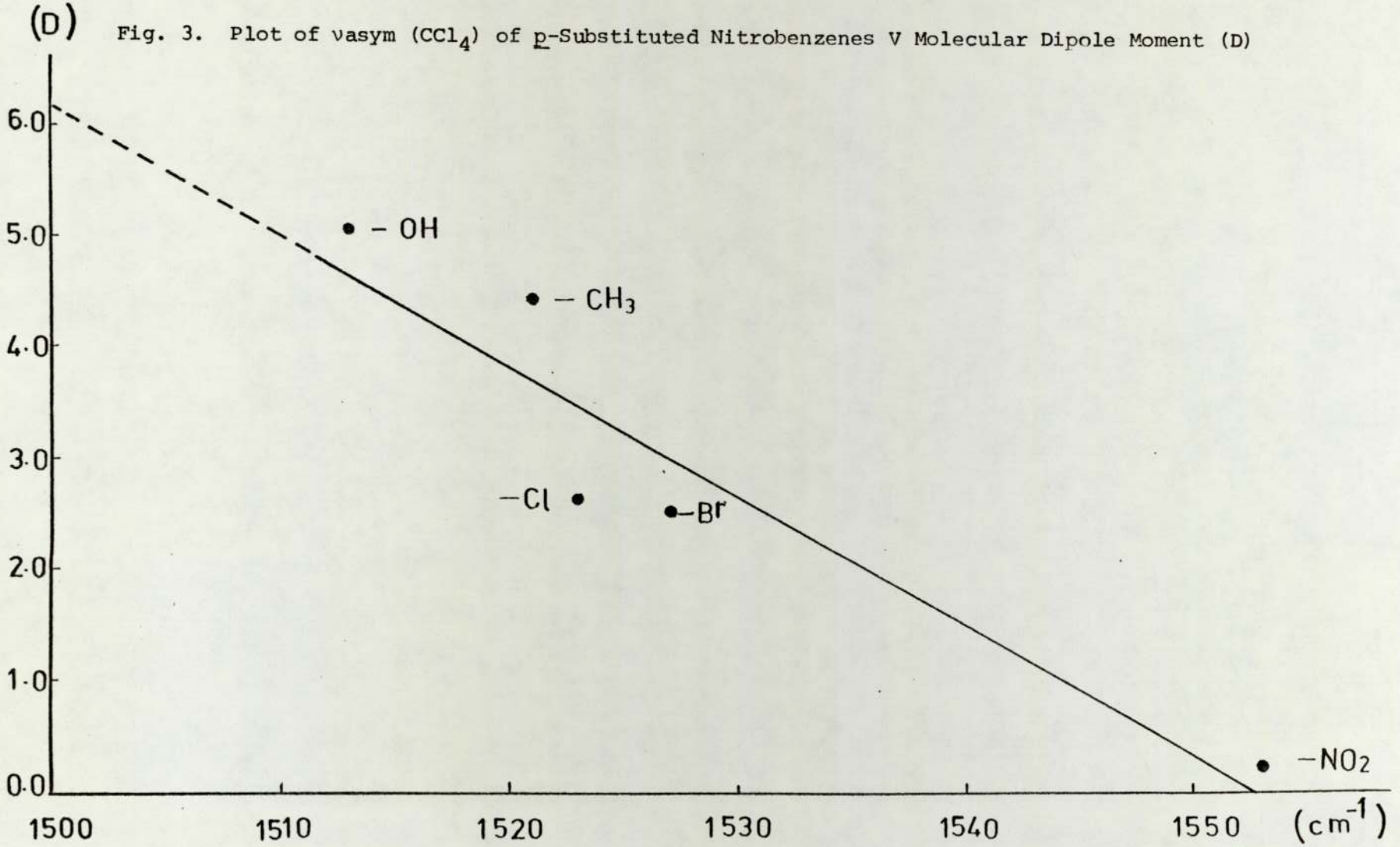


Fig. 2. Plot of νasym ($CHCl_3$) of p-Substituted Nitrobenzenes V Diple Moment (D)

Fig. 3. Plot of νasym (CCl_4) of p-Substituted Nitrobenzenes V Molecular Dipole Moment (D)



with the nitro group.

Table 6

νasym in p-Nitrobenzenes (cm^{-1})
D of p-Nitrobenzenes[89]

Compounds	νasym			D
	KBr	$CHCl_3$	CCl_4	
nitrobenzene	1524	1526	1524	3.97
p-dinitrobenzene	1545	1553	1553	0.2
p-nitrobenzoic acid	1538	1527	-	4.02
p-nitrobenzaldehyde	1536	-	-	2.4
p-nitrotoluene	1517	1515	1521	4.4
p-bromonitro benzene	1533	1529	1534	2.5
p-chloronitro benzene	1526	1521	1523	2.6
p-nitroaniline	1504	1504	-	6.3
p-nitrophenol	1512	1518	1513	5.03

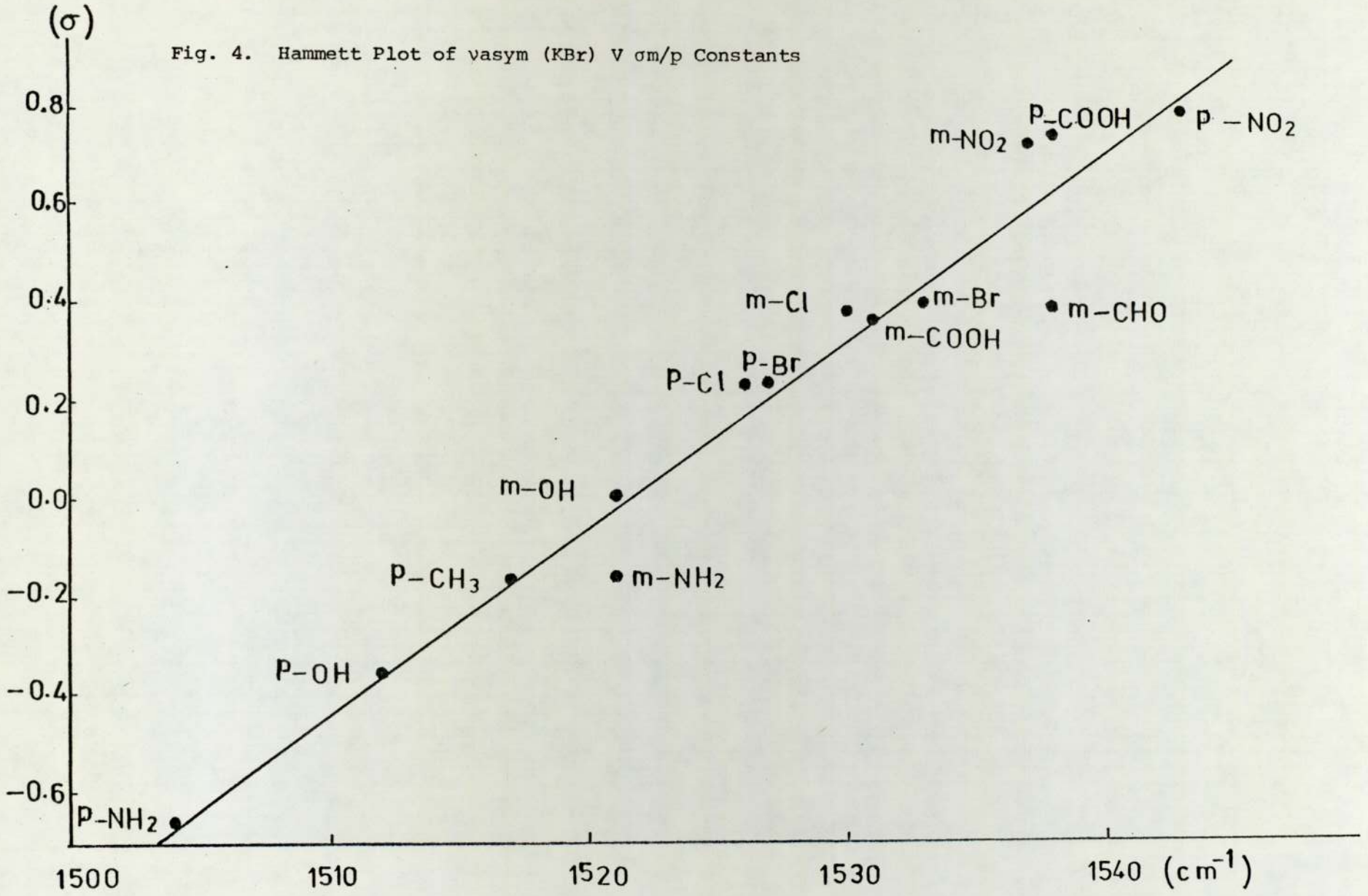
(ii) With Hammett's Constant (σ)

As a result of the sensitivity of the asymmetric band to substitution in nitrobenzenes, correlation with the Hammett substituent constant has been extensively studied.[77,87,89] Experimental data for meta- and para-substituted nitrobenzenes (Table 7) obtained in the present work has been plotted against Hammett constants (σp/m) (Figs. 4-6). From (Figs. 4-6) it is observed that deviations in meta substituents is more than in the para derivatives; the meta substituents clearly have a special effect on the overall relationship.[89]

e) Solvent effects

The intensity variations of νasym of nitro groups were almost insignificant in all solvents. A slight frequency shift was observed, however, when passing from one solvent to another (Table 3). The solid phase spectra in general show lower frequency absorptions

Fig. 4. Hammett Plot of νasym (KBr) V σm/p Constants



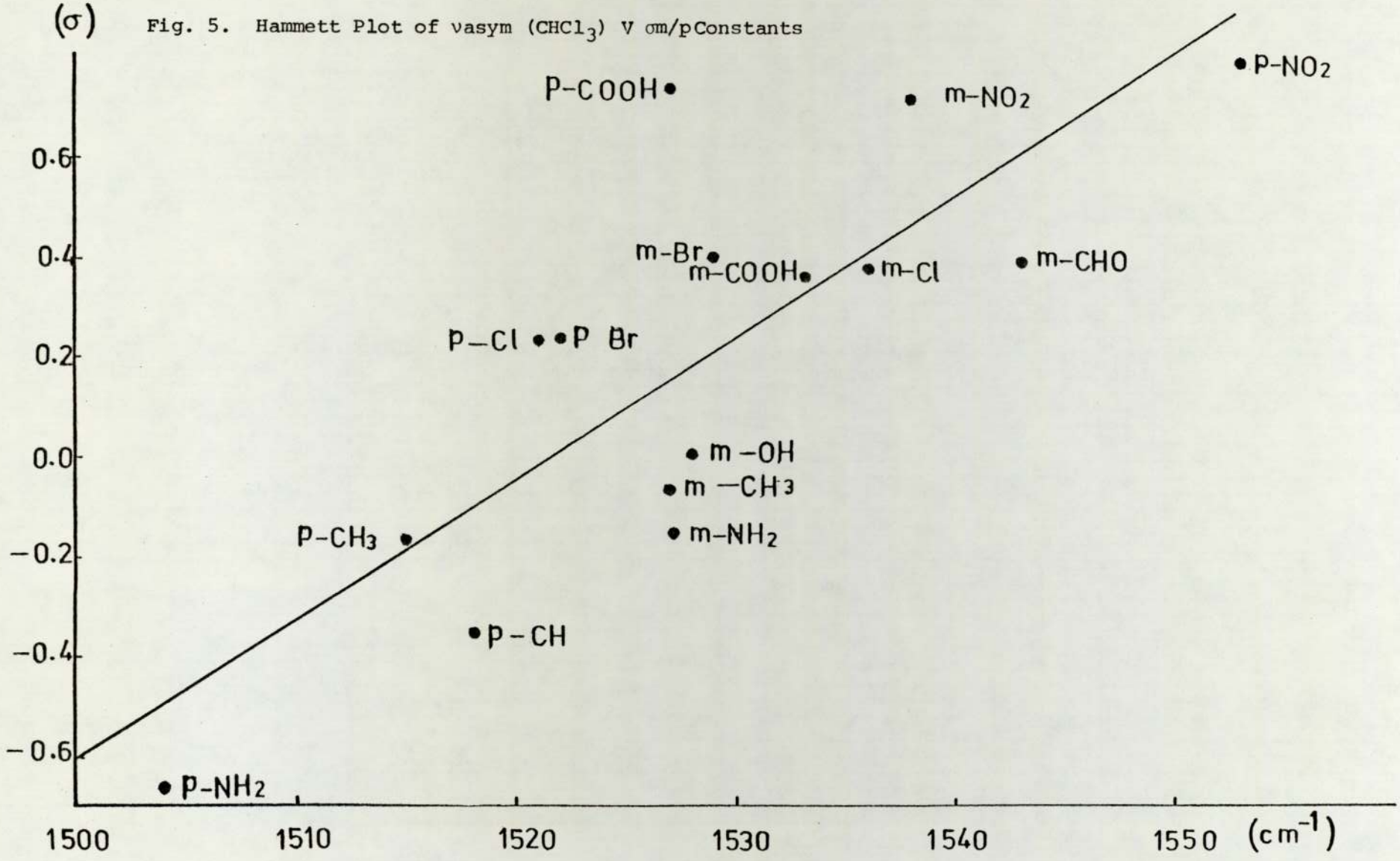


Fig. 5. Hammett Plot of νasym ($CHCl_3$) V σm/pConstants

Fig. 6. Hammett Plot of νasym (CCl_4) V σm,p Constants

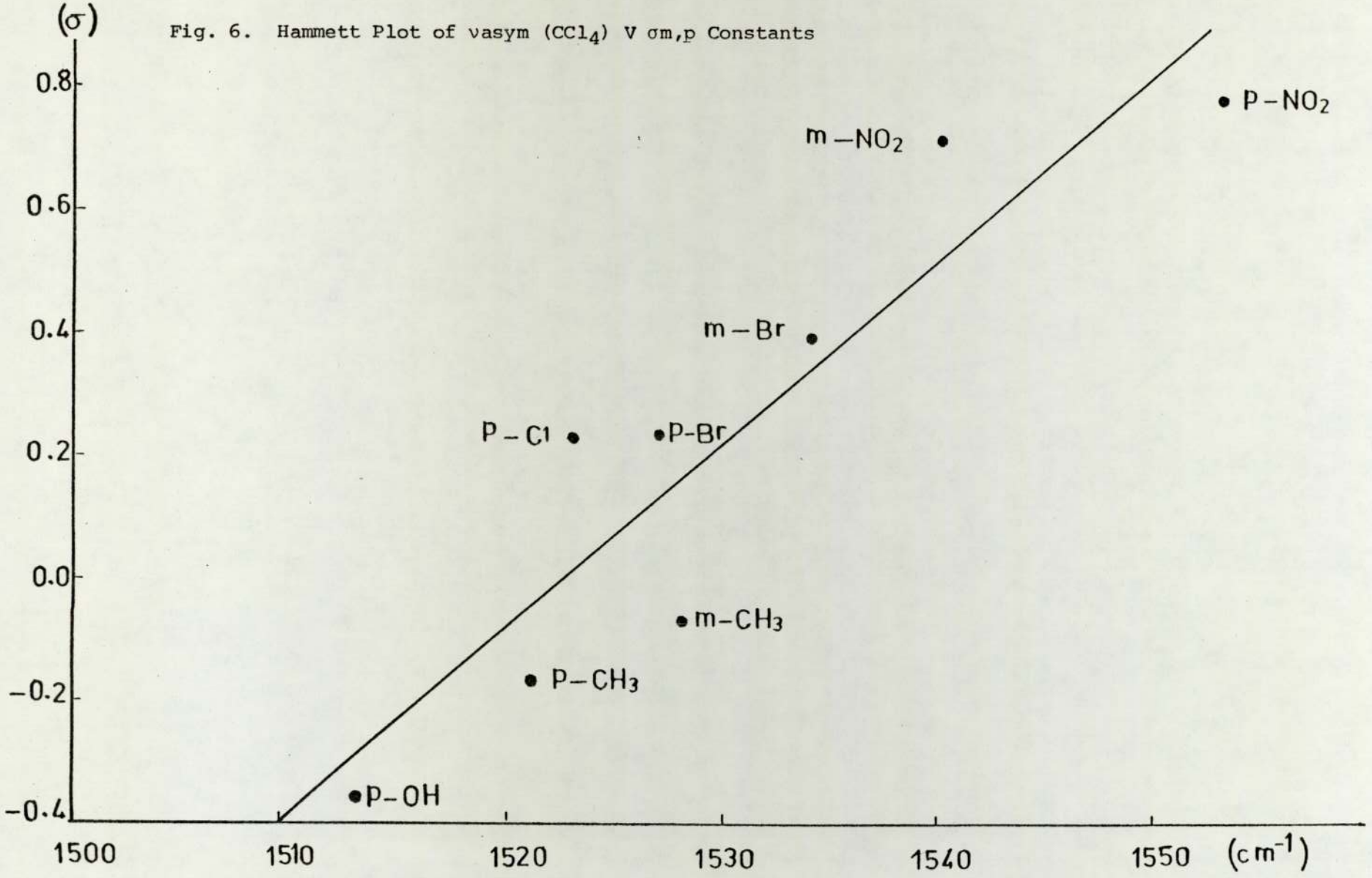


Table 7

νasym of NO_2 in Nitrobenzenes (cm^{-1})
and Hammett Constants

Compound	νasym NO_2			σm/p
	KBr	$CHCl_3$	CCl_4	
m-dinitrobenzene	1537	1538	1540	+0.71
p-dinitrobenzene	1545	1553	1553	+0.778
m-nitrobenzoic acid	1531	1533	-	+0.355
p-nitrobenzoic acid	1538	1527	-	+0.728
m-nitrobenzaldehyde	1538	1543	-	+0.381
p-nitrobenzaldehyde	1536	-	-	+1.126
m-nitrotoluene	-	1527	1528	-0.007
p-nitrotoluene	1517	1515	1521	-0.17
m-bromonitrobenzene	1533	1529	1534	+0.391
p-bromonitrobenzene	1530	1522	1527	+0.232
m-chloronitrobenzene	1530	1536	-	+0.373
p-chloronitrobenzene	1526	1521	1523	+0.227
m-nitroaniline	1521	1527	-	-0.161
p-nitroaniline	1504	1504	-	-0.66
m-nitrophenol	1521	1528	-	+0.002
p-nitrophenol	1512	1518	1513	-0.357

than the solution spectra. o-Dinitrobenzene shows an extraordinary shift (1523 cm^{-1} in solid phase shifts to 1545 cm^{-1} in chloroform). A shift to higher frequencies on changing from solid to solution phases is generally observed, and the upper and lower limits were +1 cm^{-1} to +22 cm^{-1} (Table 3). However, some para-substituted derivatives show a small shift to lower frequency (Table 8) in the solution spectra.

f) Correlation with physical constants in different solvents

The relationship of νasym against certain physical constants in different solvents as well as the solid phase is variable. Correlation coefficients in different solvents are shown in (Table 9).

Table 8

νasym NO_2 in Para-Substituted Nitrobenzenes

Compounds	νasym		
	KBr	$CHCl_3$	CCl_4
nitrobenzene	1524	1526	1524
p-nitrobenzoic acid	1538	1527	-
p-nitrotoluene	1517	1515	1521
p-bromonitrobenzene	1530	1522	1527
p-chloronitrobenzene	1526	1521	1523

Table 9

Correlation Coefficient of νasym NO_2 versus Dipole Moment and Hammett's σ Constant

Solvents	Correlation Coefficient	
	V(D)	V(σ)
KBr	0.85	0.97
$CHCl_3$	0.89	0.80
CCl_4	0.93	0.90

From (Table 9) the linearity improves in solution in the correlation with dipole moment, but correlation with σ is better in the solid phase spectra. The solvent effect is imperfectly understood and has not been extensively studied so far.

2.4 The Symmetric Stretching Frequency of the Nitro Group

This mode is subject to coupling effects which, in the aromatic series, probably involve some ring vibrations. Changes in substituents which alter the latter are therefore liable to change νsym even if there is no change in the force constants or electronic arrangement of the nitro group itself. The frequencies therefore vary in a somewhat erratic way.

a) Resonance effects

Nitrobenzenes substituted in the ortho or the para position by the +M substituents (e.g. OH or NH_2) lower the frequency of the symmetric absorption as a result of resonance effects (see 2.3a). The range (in chloroform) was -15 cm^{-1} (for a p-amino group) to -6 cm^{-1} for the corresponding o-amino group (Table 10) in relation to nitrobenzene itself.

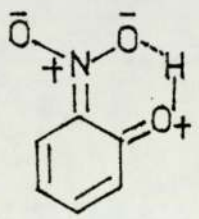
Table 10

Resonance Effects on νsym NO_2

Compounds	νsym No2		
	KBr	$CHCl_3$	CCl_4
nitrobenzene	1346	1351	1351
p-nitroaniline	1338	1336	-
o-nitroaniline	1345	1345	1342
p-nitrophenol	1345	1339	1342

b) Hydrogen bonding effects

In the case of o-nitrophenol, it appears that a strong intramolecular hydrogen bond is formed.[93] This bond represents a special case; its unusual strength is a result of the resonance contribution of the chelate structure (2.2). This causes a



(2.2)

Hydrogen bond in o-nitrophenol

pronounced shift to lower values in νsym (Table 11). νsym in o-nitrophenol is lowered by 20 cm^{-1} in comparison with nitrobenzene in the same solvent, whereas the shift in the case of o-nitroaniline

was less pronounced because of weaker hydrogen bonding. Generally speaking intra- and inter-molecular hydrogen bonding in nitro compounds is very weak.[93]

Table 11

Hydrogen Bonding Effects on νsym NO_2

Compounds	νsym NO_2		
	KBr	$CHCl_3$	CCl_4
nitrobenzene	1346	1351	1351
o-nitrophenol	1333	1331	1331
o-nitroaniline	1345	1345	1342

c) Steric effects

The symmetric stretching frequency is affected by the presence of more than one nitro group. In o-dinitrobenzene the band tends to move to a higher frequency under the influence of a steric effect and a reduction of the degree of conjugation in the aromatic system. The increase varies from +21 cm^{-1} in KBr disc to +11 cm^{-1} in $CHCl_3$ solution relative to nitrobenzene (Table 12).

Table 12

Steric Effect on νsym NO_2 in o-Dinitrobenzene

Compounds	νsym NO_2	
	KBr	$CHCl_3$
nitrobenzene	1346	1351
o-dinitrobenzene	1367	1362

d) Other effects

Symmetric subsidiary bands have been observed in p-nitrobenzenes (Table 13). Bobvich[94] noted two concentration-sensitive components of the symmetric band (1340, 1323 cm^{-1}) in the Raman spectrum of

p-nitroaniline in dioxan and assigned them to free nitro group absorptions perturbed by inter-molecular hydrogen bonds. Iogansen thought that this phenomenon was specific for p-nitroaniline and was caused by interaction of the vibration of $C-NO_2$ and $C-NH_2$ groups.[91] The same phenomenon was observed in our data (Table 13) in those para-substituted nitrobenzenes having a deactivating group in the para position as well as in p-nitroaniline.

Table 13

Splitting of νsym NO_2 in p-Nitrobenzenes

Compounds	νsym NO_2	
	KBr	$CHCl_3$
nitrobenzene	1346	1351
p-nitroaniline	1338 1309	1336 1311
p-chloronitro-benzene	1356 1342	1356 1343
p-bromonitro-benzene	1356 1342	1356 1345
p-nitrotoluene	1349 1339	1345

e) Correlation with physical constants

With Hammett's constant (σ)

The νsym NO_2 is believed to be less substituent-dependent than the asymmetric band,[89] except in its sensitivity to steric effects. Partial linear correlations with σ constants have been described, either with a break approximately at $\sigma = 0$ deviated to the positive side of σ or split into two almost parallel lines.[95] Data obtained in the present work (Figs.7-9) agreed essentially with the former view. An explanation has been based on coupling with the vibration mode of C-N so that the symmetric vibration depends on the C-N force constant. However the reason remains

Fig. 7. Hammett Plot of νsym (KBr) V σm/p Constants

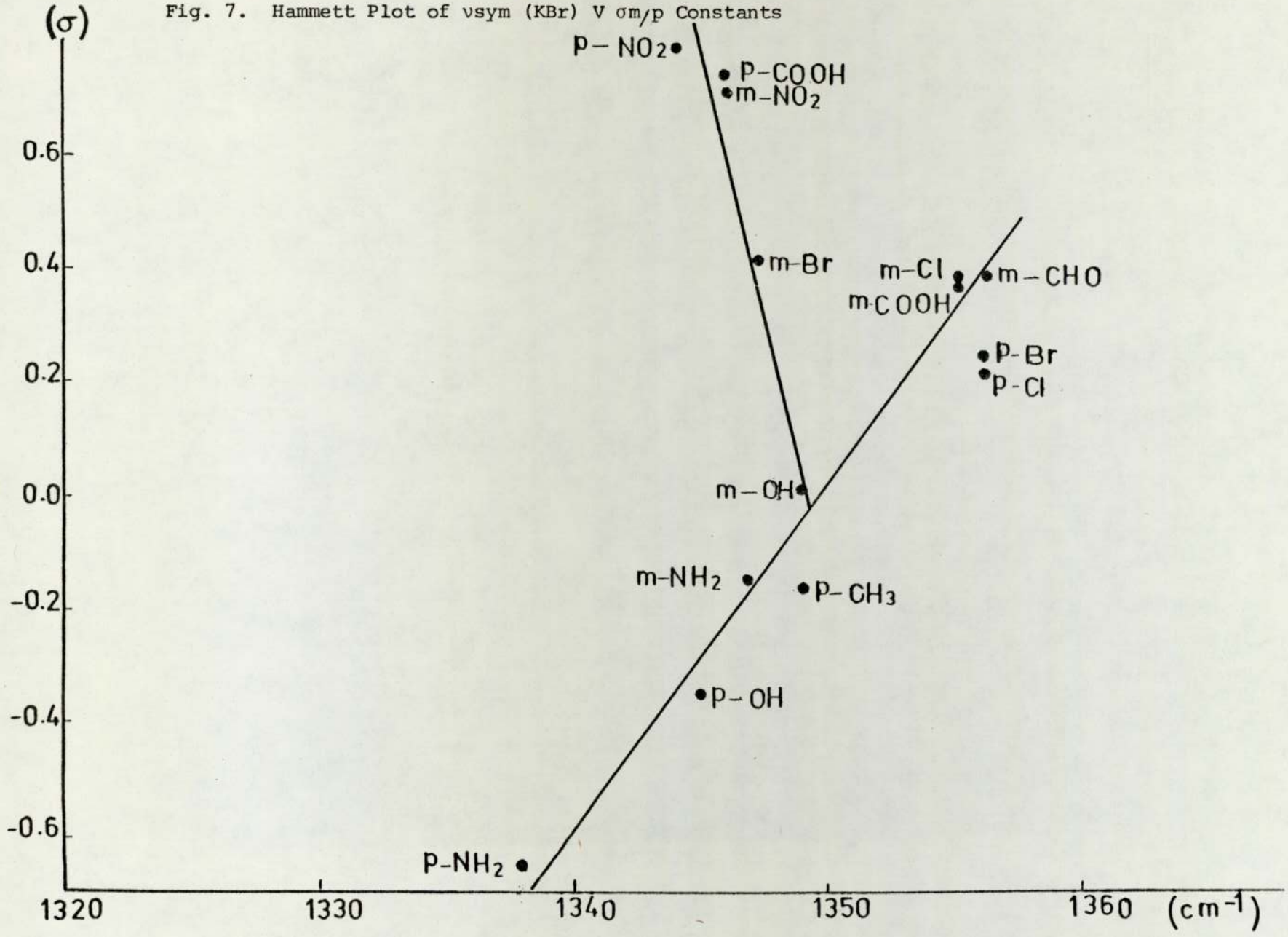


Fig. 8. Hammett Plot of νsym ($CHCl_3$) V σm/p Constants

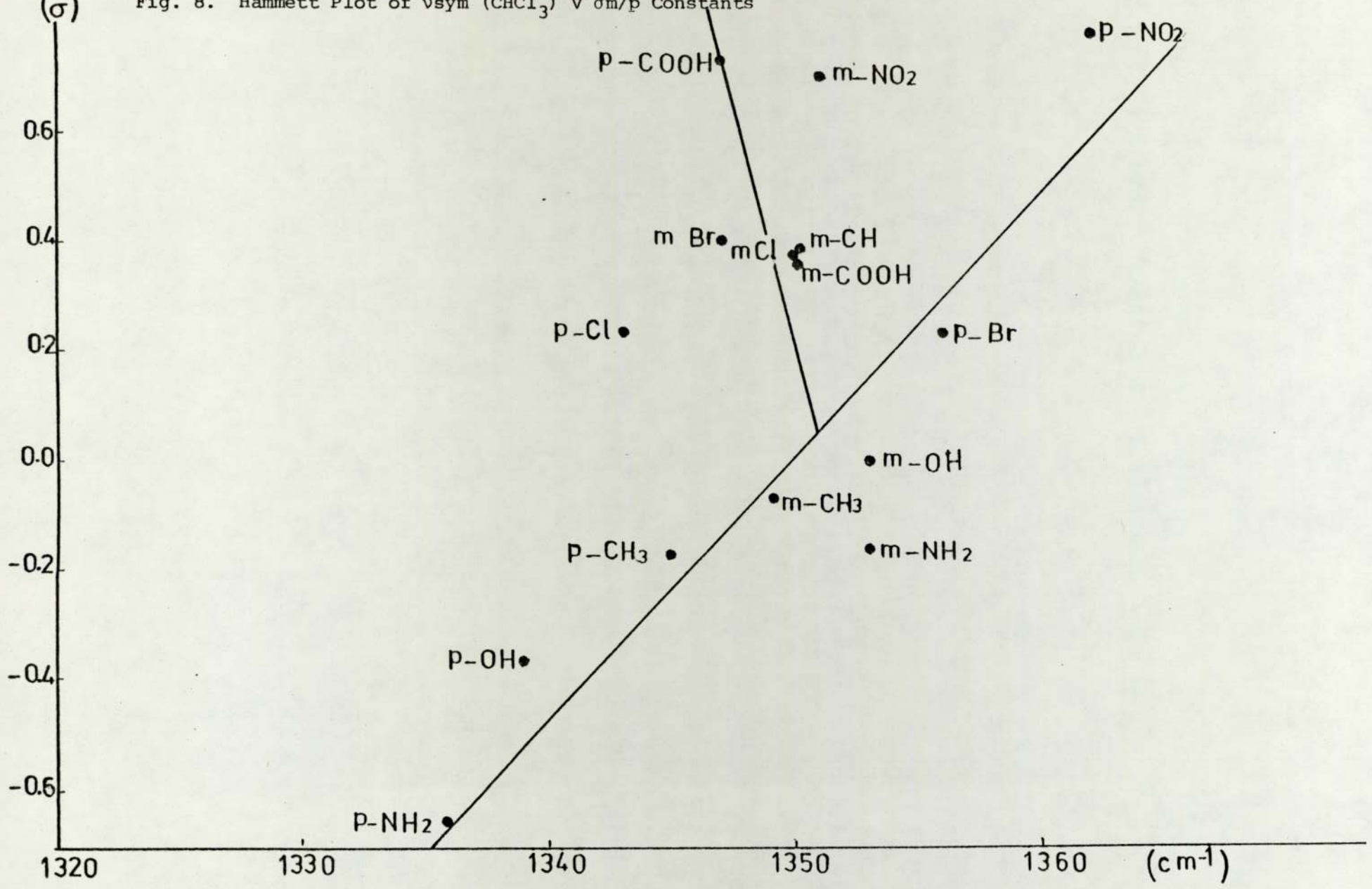


Fig. 9. Hammett Plot of νsym (CCl_4) V σm/p Constants

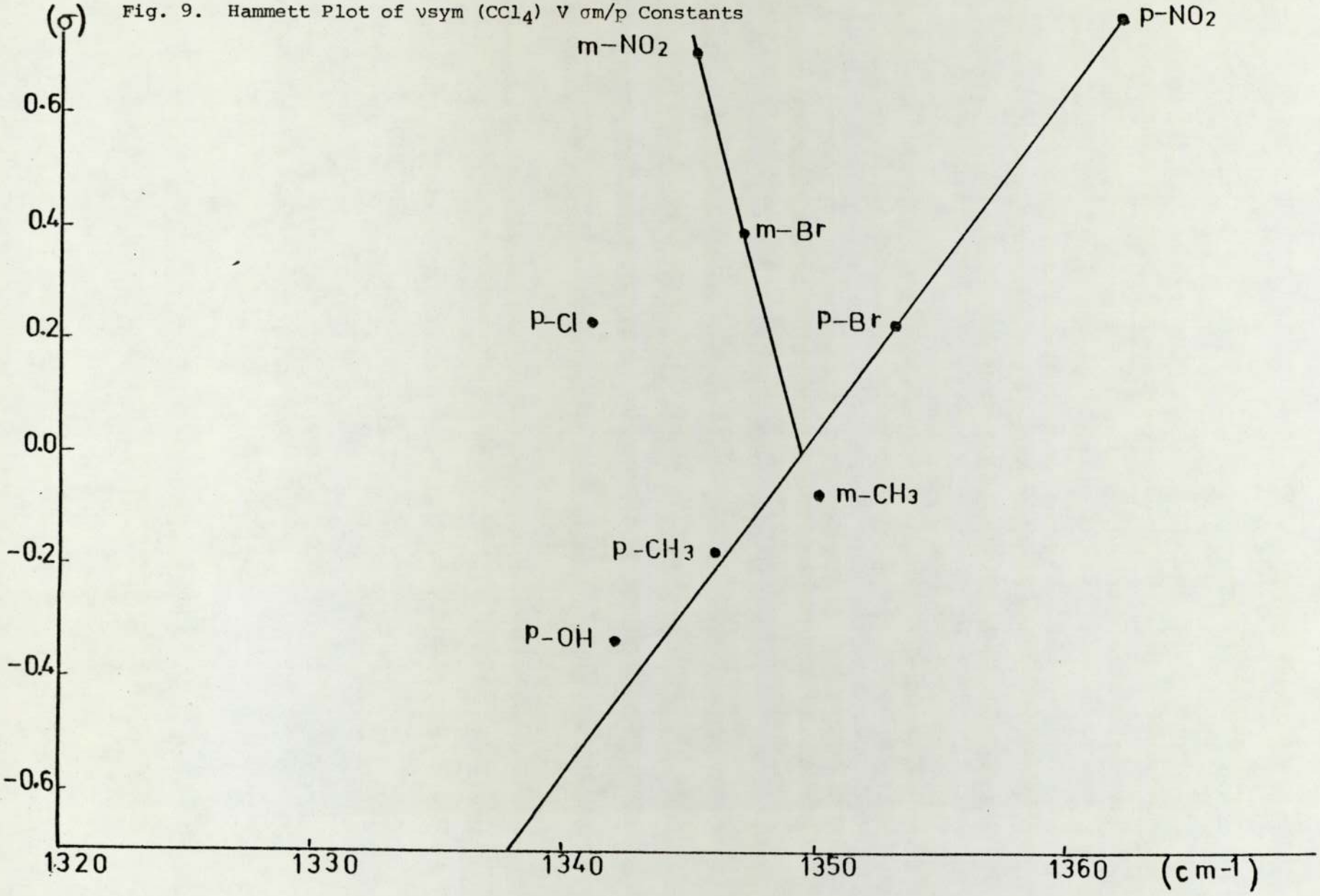


Table 14

νsym NO_2 in Nitrobenzenes cm^{-1} and Hammett's σ Constant

Compounds	νsym NO_2			$\sigma m/p$
	KBr	$CHCl_3$	CCl_4	
m-dinitrobenzene	1346	1351	1345	+0.71
p-dinitrobenzene	1344	1362	1362	+0.778
m-nitrobenzoic acid	1355	1350	-	+0.355
p-nitrobenzoic acid	1346	1347	-	+0.728
m-nitrobenzaldehyde	1356	1350	-	+0.381
p-nitrobenzaldehyde	1343	-	-	+1.126
m-nitrotoluene	-	1349	1350	-0.007
p-nitrotoluene	1349	1345	1346	-0.17
m-bromonitrotoluene	1347	1347	1347	+0.391
p-bromonitrobenzene	1356	1356	1353	+0.232
m-chloronitrobenzene	1355	1350	-	+0.373
p-chloronitrobenzene	1356	1343	1341	+0.227
m-nitroaniline	1347	1353	-	-0.161
p-nitroaniline	1338	1336	-	-0.66
m-nitrophenol	1349	1353	-	-0.002
p-nitrophenol	1345	1339	1342	-0.357

obscure. Exner[89] explained that the interplay of inductive and mesomeric effects could be important; the former effects prevail in substituents with a positive σ value which influences the C-N bond more. In addition the "meta effect" could possibly be a reason for the unexplained meta deviation.[89]

f) Correlation with asymmetric frequencies

The relationship of νsym to νasym has been investigated. Figs (10-12) reveal a rather poor relationship with a large deviation from a straight line.

g) Solvent effects

The symmetric frequency shifts variably to lower values on changing from solid phase to solution spectra. A few compounds,

Fig. 10. Plot of νasym (cm^{-1}) V νsym (cm^{-1}) in KBr

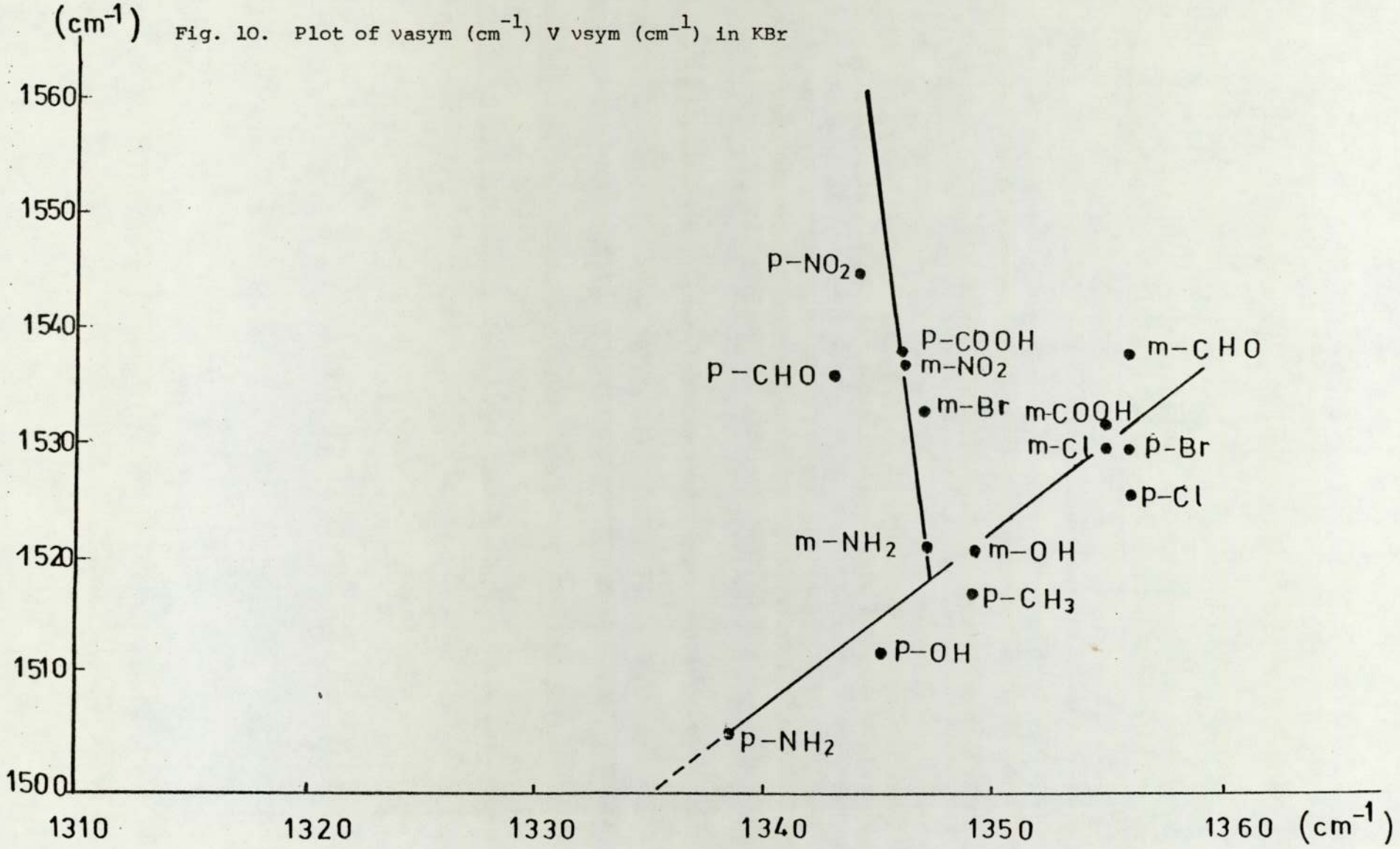


Fig. 11. Plot of νasym (cm^{-1}) V νsym (cm^{-1}) in $CHCl_3$

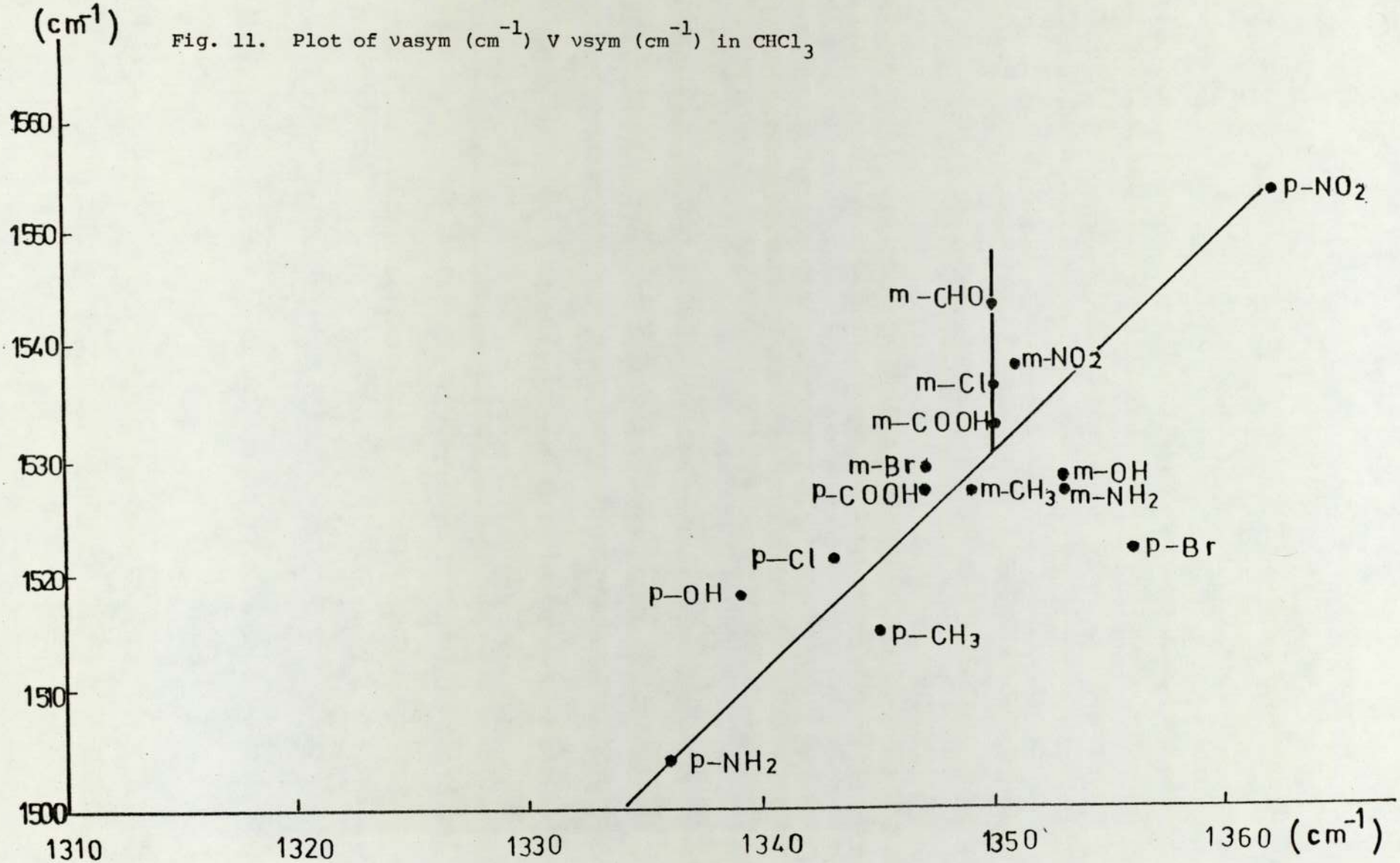
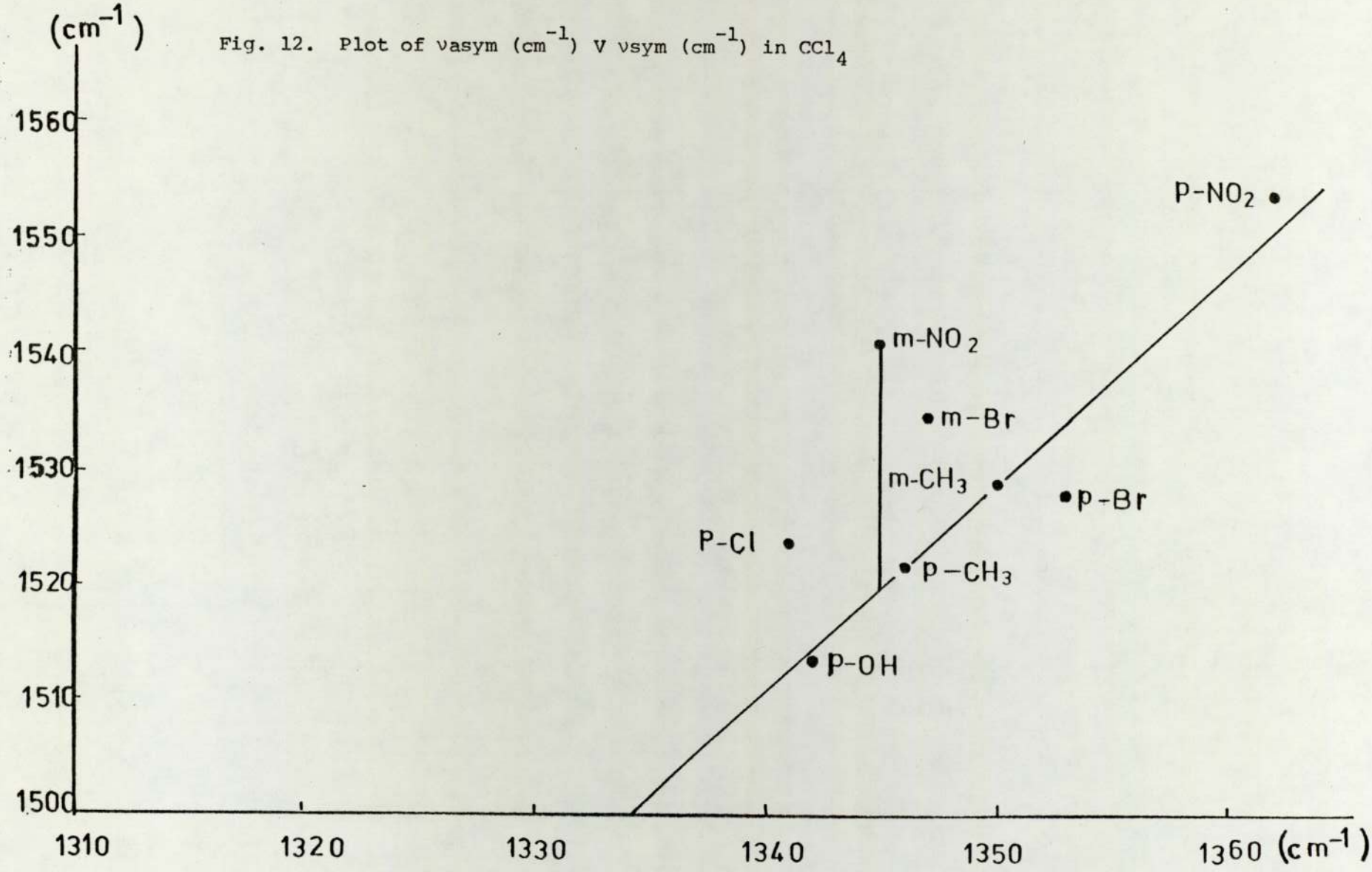


Fig. 12. Plot of νasym (cm^{-1}) V νsym (cm^{-1}) in CCl_4



mainly meta- and para-substituted derivatives, show a shift to higher frequencies (Table 4). The subsidiary band in p-nitrotoluene (at 1339 cm^{-1}) in KBr disc, disappeared in the solution spectra in $CHCl_3$ (Table 13).

2.5 Infra red Spectroscopy of Nitrobenzamides

Introduction

Characteristic bands in the infra-red spectra of primary amides are those in the region 3200-3400 cm^{-1} associated with the N-H stretching absorption and near 1650 cm^{-1} attributable to C=O stretching and N-H deformation of carbonyl and amino groups respectively. The strongest and most characteristic bands of secondary amides lie between 1500 cm^{-1} and 1700 cm^{-1}.[96] A band centered near 1660 cm^{-1} is due mainly to C=O stretch and one near 1550 is due to a composite vibration involving both stretching of the C-N bond and bending of N-H. Randall et al [97] gave details of the band position in mono-substituted amides; the frequency range was found to be 1570-1515 cm^{-1} for compounds examined in the solid state. A considerable proportion of these absorptions occur near the mean value of 1540 cm^{-1} but in solution the band shifts towards lower frequency. For example in N-methylacetamide, the frequency shift is from 1565 cm^{-1} as a thin film to 1534 cm^{-1} in chloroform solution.[98] Clearly, the bands mentioned above attributable to amide absorptions will appreciably interfere with a recognition of the characteristic nitro absorptions. Precise values for the frequencies of the nitro group absorptions of some aromatic nitrobenzamides in KBr discs and $CHCl_3$ solutions are listed in Table 15.

Flett[99] observed that the amide band at 1540 cm^{-1} was less intense in the nitroamides because of the competing absorption of the nitro group. In benzamide (Fig. 13), there is no band at 1540 whereas in o-nitrobenzamide (Fig. 14) the nitro group bands (1520 cm^{-1} and 1359 cm^{-1}) are clearly identifiable. But

Fig. 13

Benzamide in KBr

Fig. 14

o-Nitrobenzamide in KBr

Fig. 15

Benzanilide in KBr

Fig. 16

o-Nitrobenzanilide in KBr

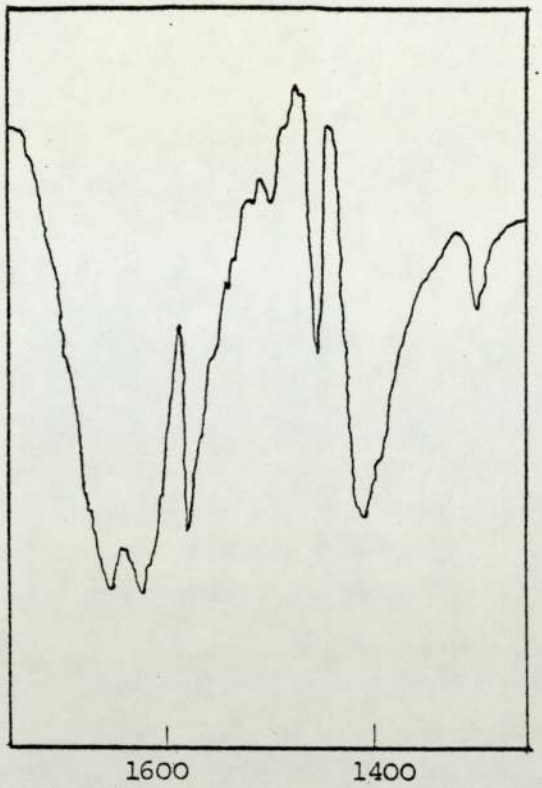


Fig. 13

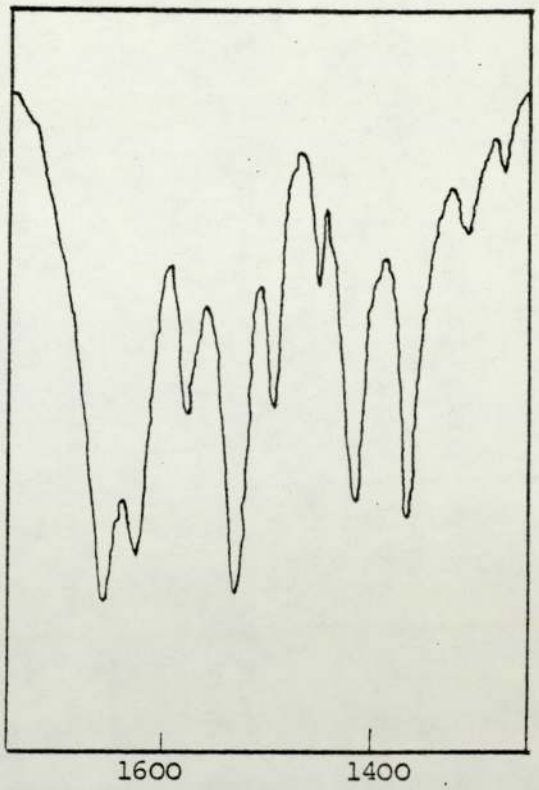


Fig. 14

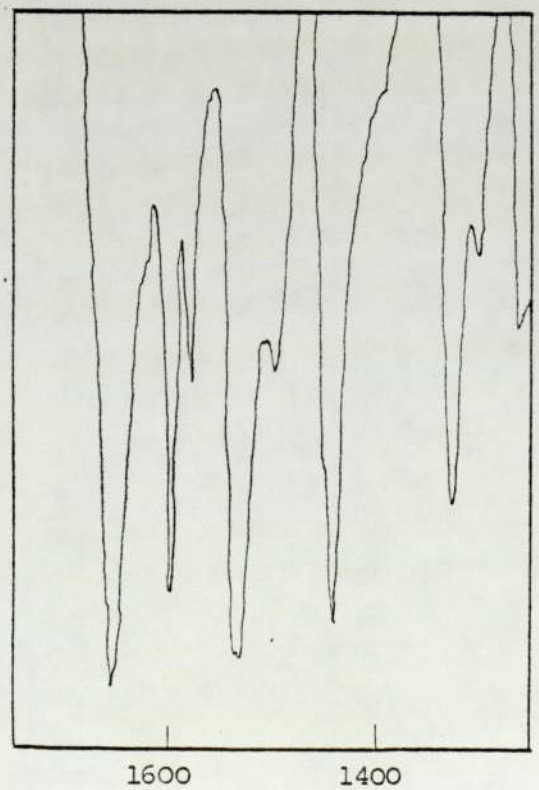


Fig. 15

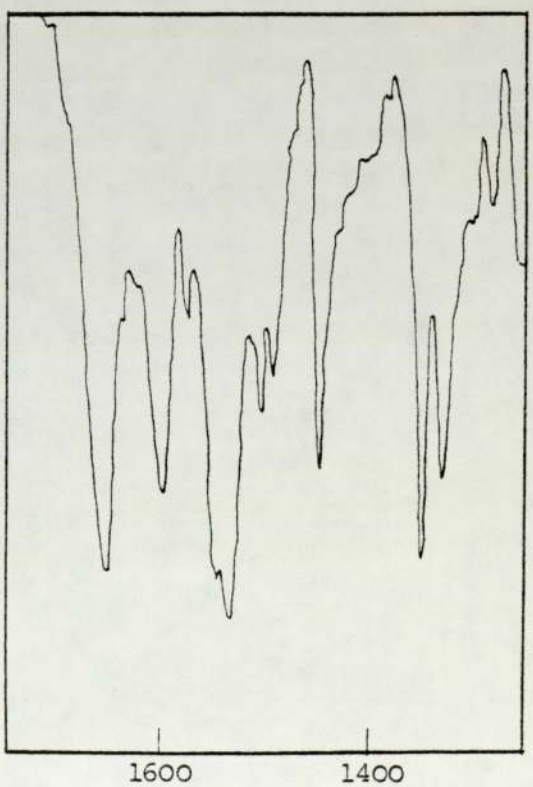
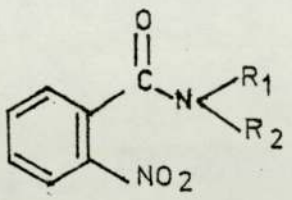


Fig. 16

there is often more than one peak in both 1350 cm^{-1} and 1530 cm^{-1} ranges. The cases of the solid phase spectra of the secondary amides benzanilide (Fig. 15) and o-nitrobenzanilide (Fig. 16) are examples, and it is difficult to identify which are the nitro stretching frequencies in most of the investigated amides.

Table 15

Frequencies of Nitro Group Stretching Vibrations



Compounds		νasym cm^{-1}		νsym cm^{-1}	
R_1	R_2	KBr	$CHCl_3$	KBr	$CHCl_3$
H	H	1520	1523	1359	1349
H	Me	1526	1531	1349	1351
H	Et	1522	1529	1361	1349
H	benzyl	1526	1533	1354	1350
H	phenylethyl	1533	1526	1354	1349
H	2-naphthyl	1533	1526	1354	1353
H	phenyl	1529	1528	1346	1348
H	o-tolyl	1522	1533	1350	1350
H	mesityl	1518	1522	1350	1351
H	o-nitrophenyl	1530	1529	1343	1350
H	p-nitrophenyl	1527	1529	1356	1351
H	o-cyanophenyl	1522	1526	1349	1352
H	p-cyanophenyl	1526	1528	1349	1347
H	p-methoxyphenyl	1530	1528	1343	1349
H	m-tolyl	1528	1533	1350	1349
Me	phenyl	1522	-	1350	-
Et	phenyl	1521	1533	1350	1349

Solution spectra of nitrobenzanilides are easier to interpret. Solution spectra in chloroform or the polar solvent dimethylsulphoxide

always show a single peak in both of the nitro group ranges (1350 and 1530 cm^{-1}). For example, the solid phase spectrum of N-methyl-o-nitrobenzamide (Fig. 17) in KBr displays multiple peaks in the 1350 cm^{-1} range which makes it very difficult to identify the nitro group stretching band: on the other hand, in the solution spectrum ($CHCl_3$) of the same compound (Fig. 18), the number of peaks in this range is reduced to one (1351 cm^{-1}) which is easily identified as the nitro group stretching frequency. Clearly the amide bands in the 1350 cm^{-1} region can be attributed to inter-molecularly hydrogen bonded amide groups. These absorptions disappear, as expected, in solution spectra leaving the nitro absorptions unambiguously defined. The nitro group, on the other hand, is not involved to any appreciable extent in inter- or intra-molecular hydrogen bonds. N-Ethyl-o-nitro-benzamide (Fig. 19) (KBr disc), also shows the problem of multiple peaks in the 1530 range, while the solution spectrum in chloroform (Fig. 20) shows only the typical nitro absorption at 1529 cm^{-1}.

As an alternative approach to resolve the multiplicity of peaks that appear in the nitro group range the use of dimethylsulphoxide was examined. This is a powerful, polar solvent capable of forming inter-molecular hydrogen bonds. It considerably simplifies the infra-red spectrum of N-p-methoxyphenyl-2-nitrobenzamide, although there are still two peaks in the 1530 cm^{-1} region in the spectrum in dimethylsulphoxide (Fig. 22) compared to that of the spectrum in KBr (Fig. 21).

A completely novel approach to unequivocally identifying the nitro group absorptions which occur in what has been described as the "psychiatric" region of the spectrum[100] was then investigated. This involved an examination of the spectra of nitro compounds

Fig. 17

N-Methyl-o-nitrobenzamide
in KBr

Fig. 18

N-Methyl-o-nitrobenzamide
in $CHCl_3$

Fig. 19

N-Ethyl-o-nitrobenzamide
in KBr

Fig. 20

N-Ethyl-o-nitrobenzamide
in $CHCl_3$

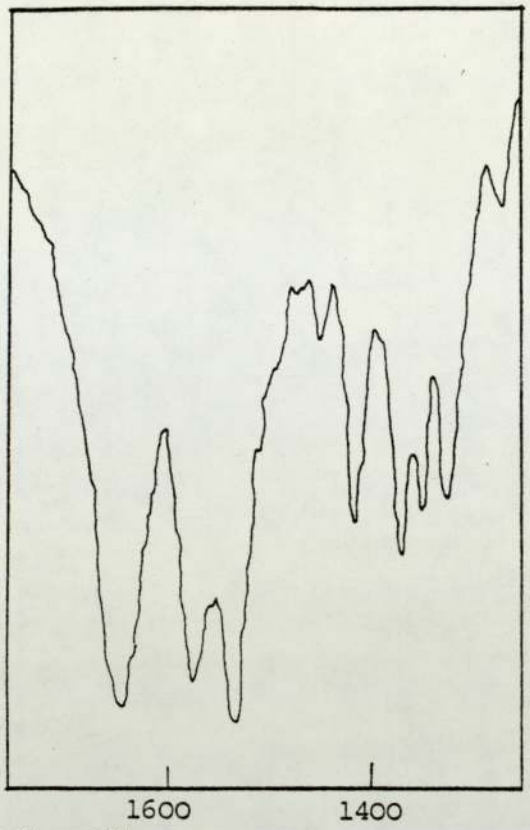


Fig. 17

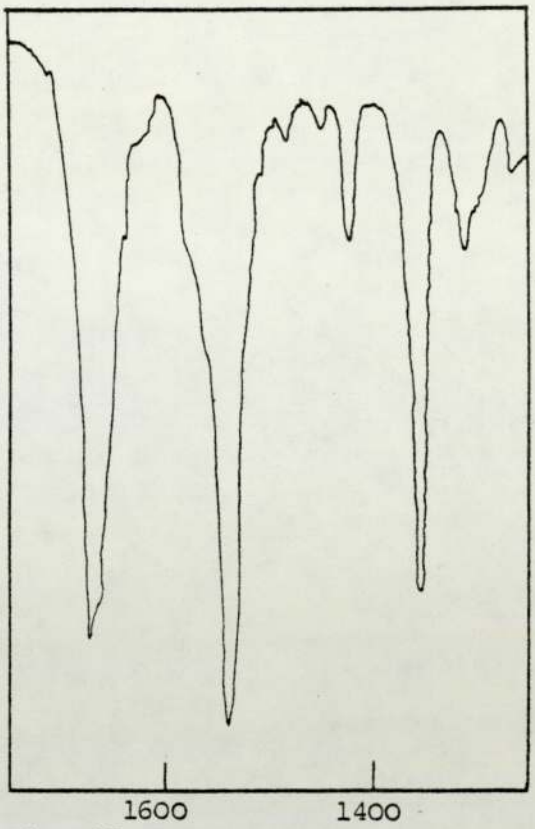


Fig. 18

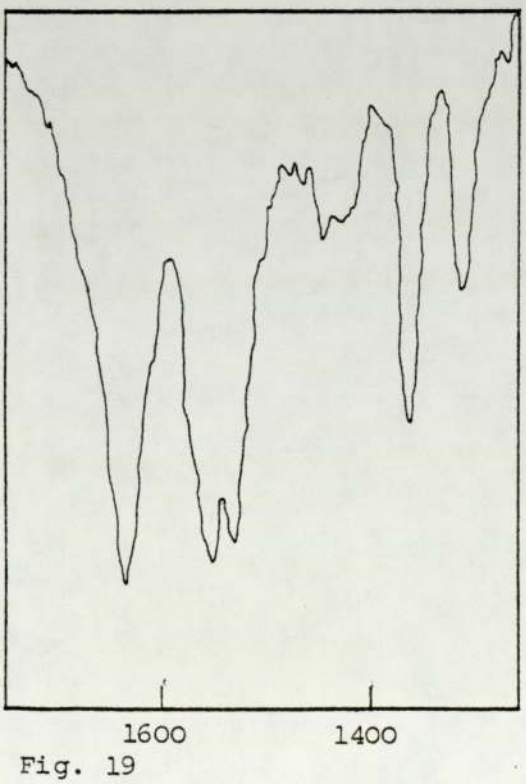


Fig. 19

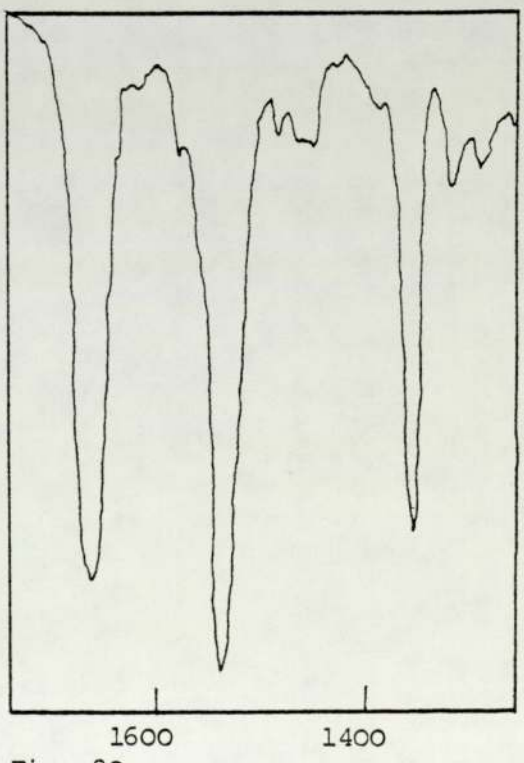


Fig. 20

Fig. 21

N-4-methoxyphenyl-o-nitrobenzamide
in KBr

Fig. 22

N-4-Methoxyphenyl-o-nitrobenzamide
in DMSO

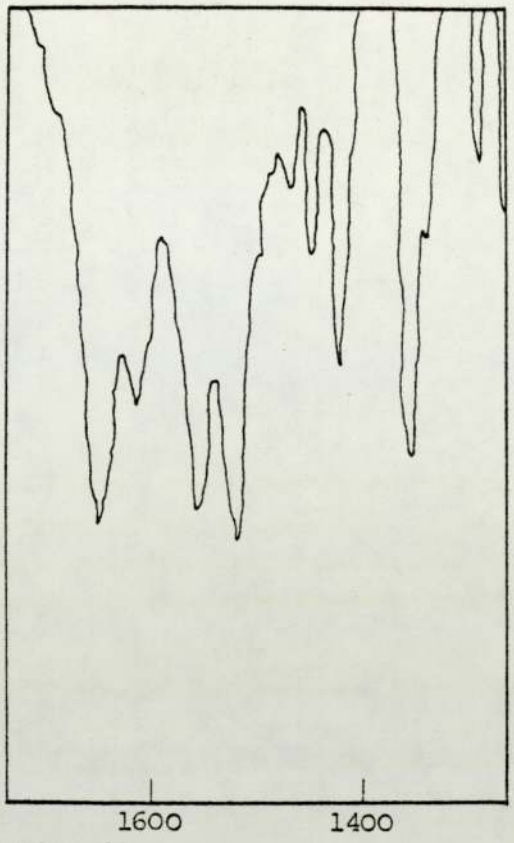


Fig. 21

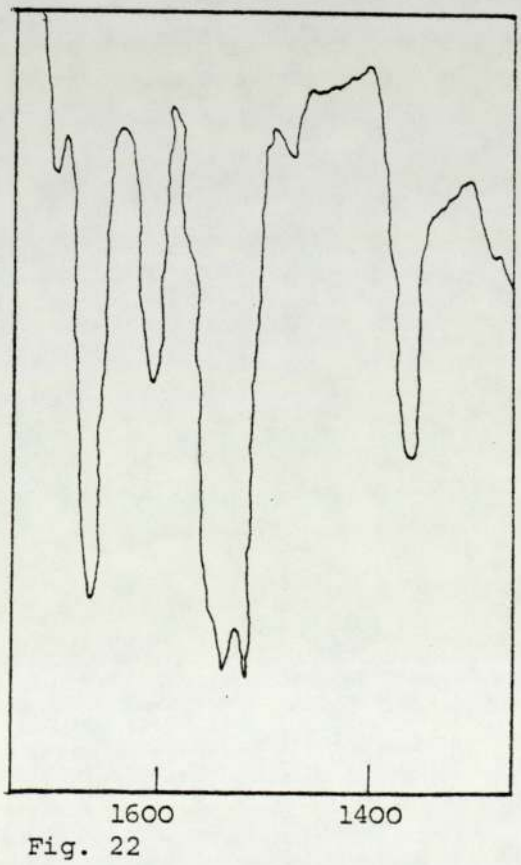


Fig. 22

in triethyl phosphite, a reagent known to effect de-oxygenation of nitro groups under vigorous conditions.

De-oxygenation of Nitro Compounds

De-oxygenation of nitro compounds by trivalent phosphorous compounds has been widely reported.[101] Various trivalent phosphorous compounds have been used as de-oxygenating agents (Table 16) but in general triethyl- or tri-methyl phosphite either alone or in an inert solvent, appear to be the reagents of choice.

Table 16

Trivalent phosphorus de-oxygenating agent* (X_3P)

X_3P	Temp.	$t_{\frac{1}{2}}$ (min)
$(Pr^iO)_3P$	143.5°	63
$(EtO)_3P$	145	50
$(EtO)_3P$ + MeN.CHO (equimolar)	144	97
$(Eto)P(NEt_2)_2$	121	
$(Et_2N)_3P$	111	41
$(EtO)_2PMe$	61	154

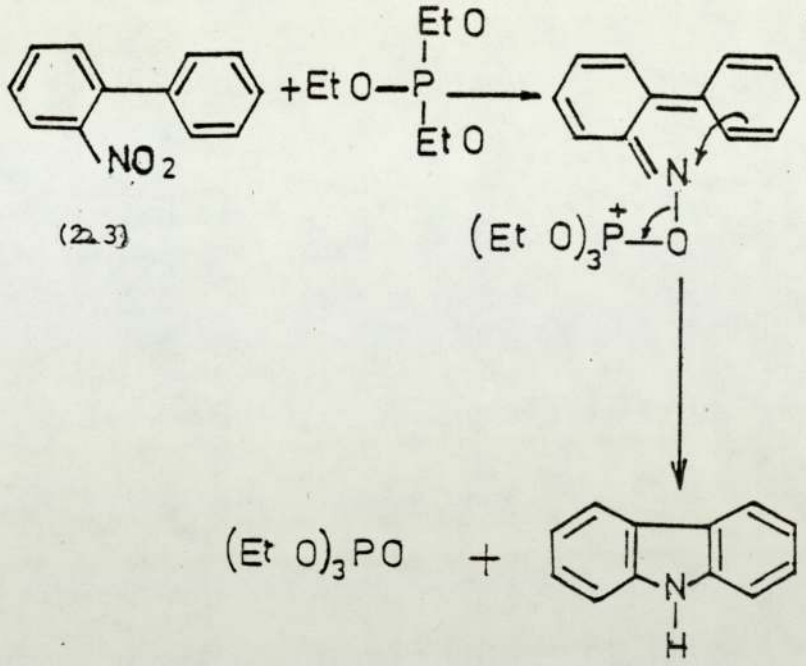
Many heterocyclic systems have been synthesised by de-oxygenation of suitably substituted ortho nitro compounds. Often forcing conditions are required to bring about cyclisation (Table 16).

Examples of Cyclisation of o-Nitro Compounds

The triethyl phosphite induced reduction of 2-nitrobiphenyls has been reported.[102,103] Reaction of 2-nitrobiphenyl (2.3) with

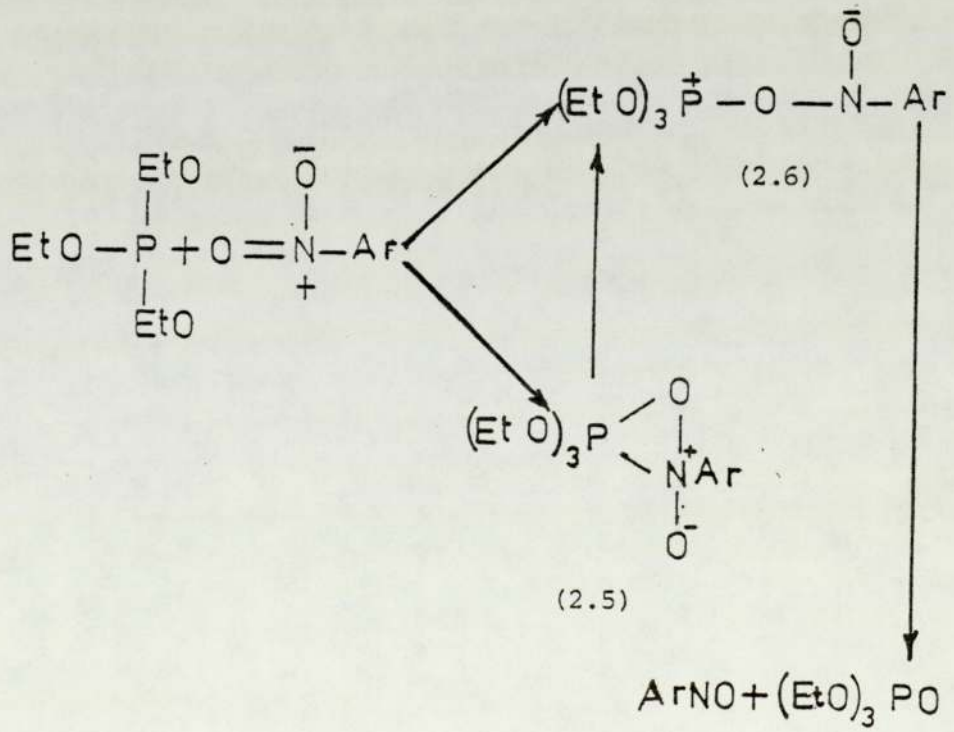
* From the study of the relative efficiencies of de-oxygenation of 2-nitrobiphenyl in excess trivalent phosphorous reagent (15-20 moles).

(scheme 3)



(2.3) (2.4)

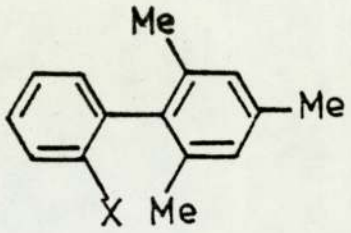
(scheme 4)



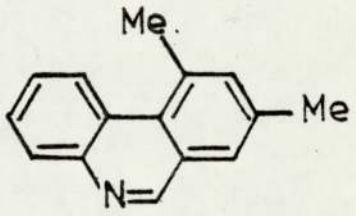
(2.6) (2.5)

a slight excess over two equivalents of boiling triethyl phosphite yields carbazole (2.4). The exact mechanism of this reaction is still in doubt. The main point of interest concerning the mechanism of the ring closure centres, however, on the possibility of either the participation of a nitrene - formed from the intermediate nitrosocompound - or by a more direct route (scheme 3). The question of the point of initial attack by the phosphorous atom is also open to discussion. It is possible that initial attack by the phosphite occurs at the more positively polarised nitrogen atom, followed by rearrangement *via* a three membered cyclic intermediate (2.5) to a dipolar structure (2.6) in which the phosphate leaving group is latent. Alternatively the latter may be formed by direct attack on the oxygen atom (scheme 4). Evidence for the participation of a nitrene intermediate in this type of reaction is usually taken to be the occurrence of abstraction and insertion reactions with C-H bonds.[104] Thus the formation of 8,10-dimethylphenanthridine (2.8), 1,3,5-trimethylcarbazole (2.9), and 2'-amino-2,4,6-trimethylbiphenyl (2.10) has been attributed to the participation of a nitrene in the decomposition of 2'-azido-2,4,6-trimethylbiphenyl (2.7; $X = N_3$) in hexadecane at 230°.[105]

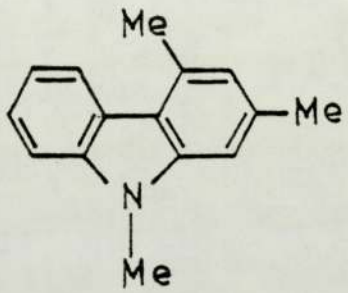
Interaction of 2'-nitro-2,4,6-trimethylbiphenyl (2.7; $X = NO_2$) with excess of triethyl phosphite afforded the amine (2.10) and triethyl N-(2',4',6'-trimethylbiphenyl-2-yl)phosphorimidate (2.11).[106,107] Products of insertion (2.8) and (2.9), were not detected however. These results are compatible with the intermediacy of a discriminating nitrene which abstracts hydrogen to give (2.10) and couples with triethyl phosphite, present in excess, to give (2.11), rather than undergo the presumably less energetically



(2.7)



(2.8)



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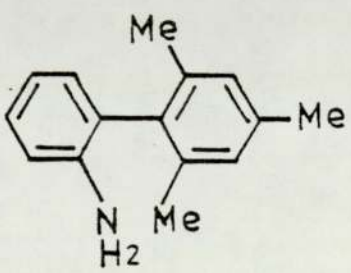
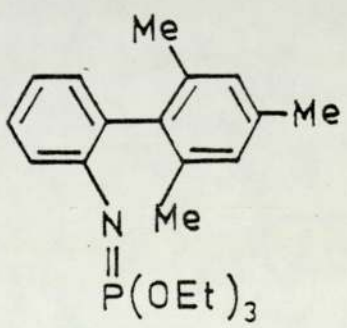
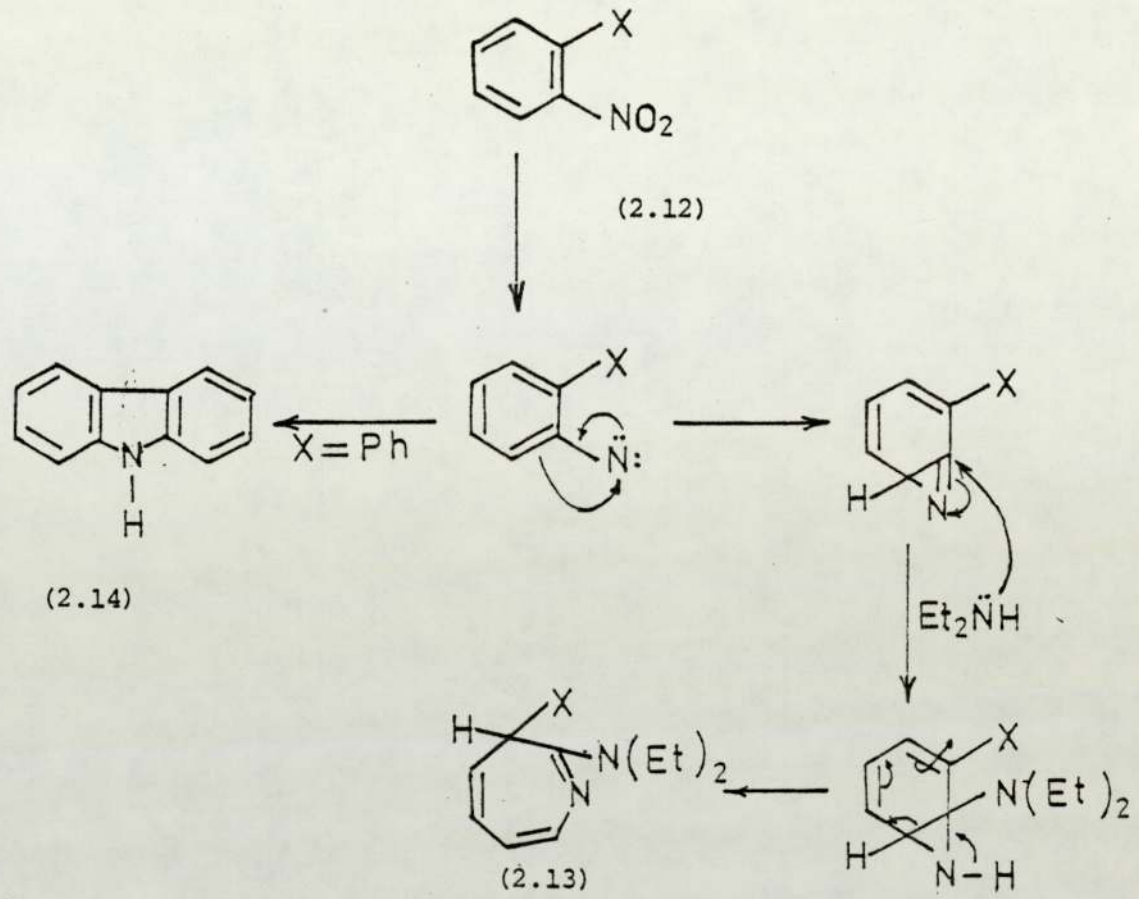


(2.10)

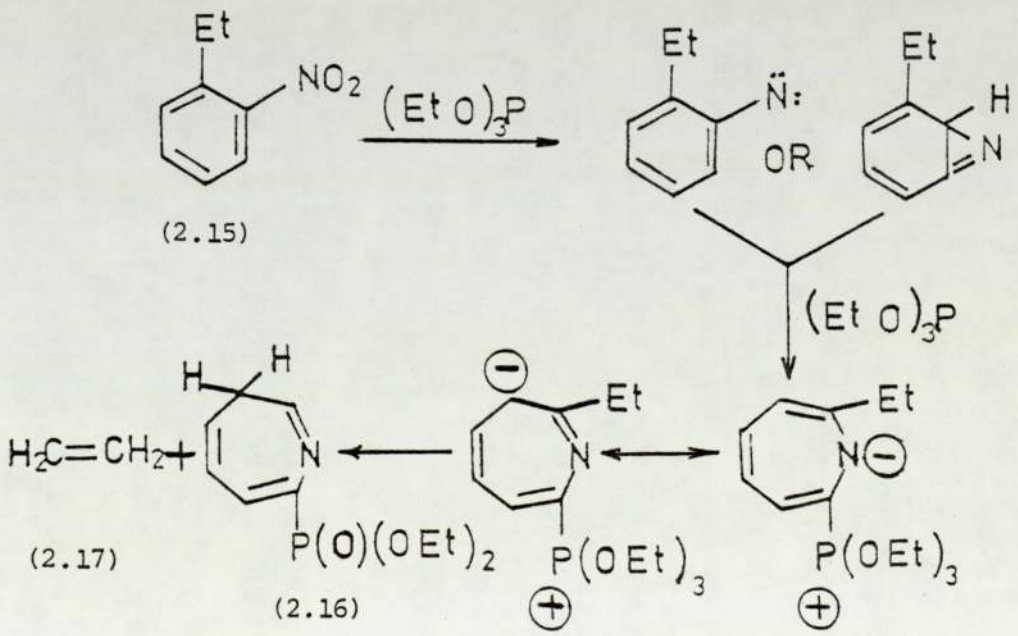


(2.11)

(scheme 5)



(scheme 6)



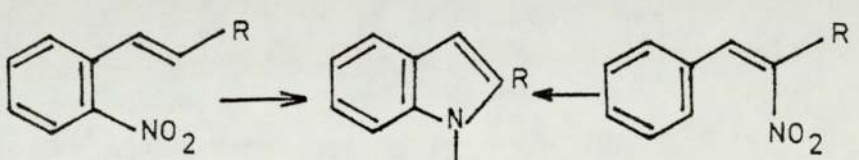
favourable insertions to give (2.8) and (2.9).

In this connection, reaction of diethylmethylphosphonite, a very reactive trivalent phosphorous reagent, with nitrobenzene (2.12; X = H) and with 2-nitrobiphenyl (2.12; X = Ph) in excess of diethylamine[108] has been shown to give the 3H-azepines (2.13; X = H and Ph) respectively (scheme 5), thus indicating a strong parallel between these reactions and those of phenylazide decompositions in diethylamine.[109] In addition to the latter azepine, carbazole (2.14) was also formed. This raises the possibility that carbazole is also formed from a nitrene intermediate in this reaction.

Ring expansion has also been reported in the de-oxygenation of o-ethylnitrobenzene (2.15) which with excess boiling triethyl phosphite, gives 2-ethyl-3-H-azepine-7-ylphosphonate (2.16) and ethylene[110,111] (2.17) (scheme 6).

By analogy with the cyclisation of 2-nitrobiaryl to carbazole, o-nitrostyrenes (2.18) or nitrostilbenes (2.19) give rise to indoles (2.20) (scheme 7; R = Ph)

(Scheme 7)

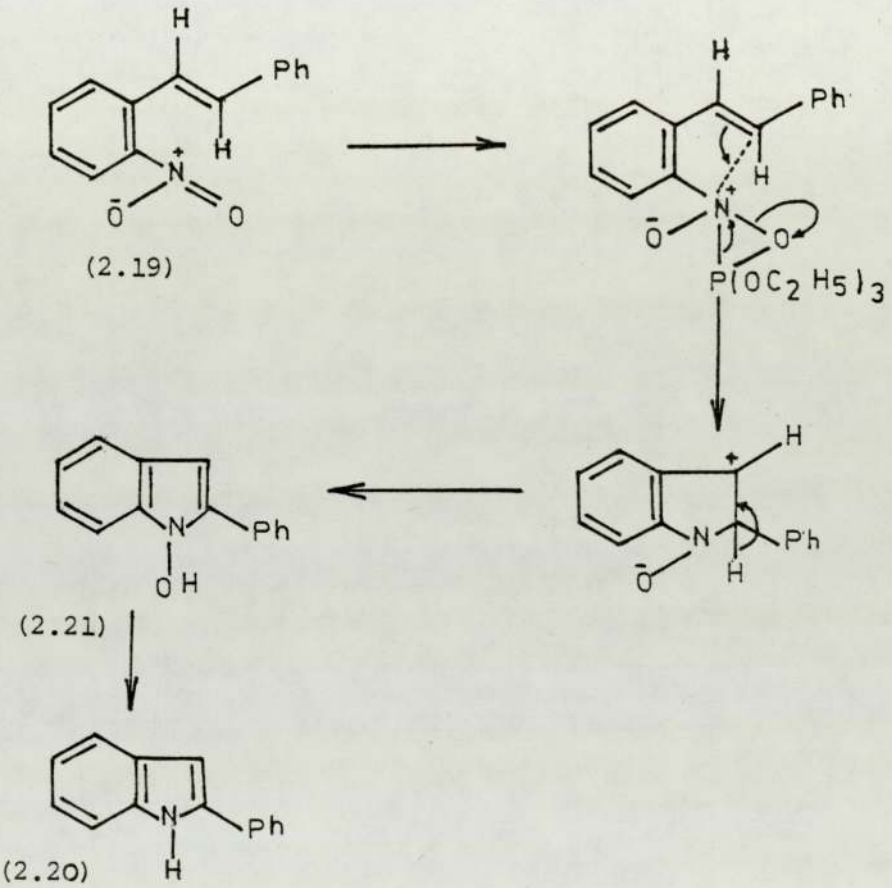


(2.18) (2.20) (2.19)

It is tempting to extend the analogy between the reductive cyclisation of nitrobiaryls to carbazoles, and o-nitrostyrene derivatives to indoles to include a common mechanisms, but some results reported by Sundberg[111] in the course of an extension of this indole synthesis indicate that this may not be valid. Sundberg[111] postulated 1-hydroxy-2-phenylindole (2.21) as an

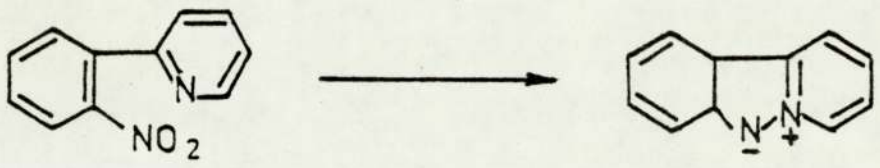
intermediate, and even isolated this compound, from partial de-oxygenation of the stilbene (2.19): on further heating with triethyl phosphite the hydroxyindole gave (2.20) (scheme 8).

(scheme 8)

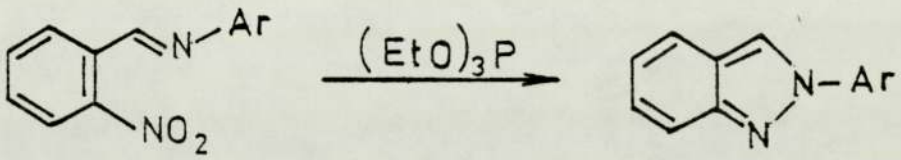


The demonstration that 1-hydroxy-2-phenylindole is an intermediate indicated that there may be an important mechanistic difference between this reaction and the corresponding reductive cyclisation of 2-nitrobiaryls.

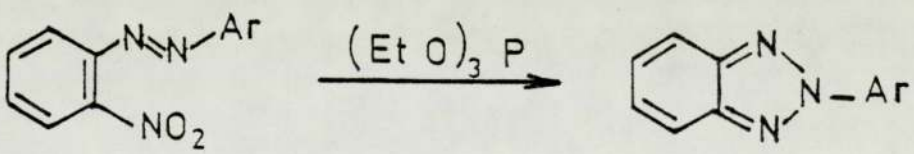
Following the report[103,112] that an electrophilic nitrogen species (a nitrene) is an intermediate in the de-oxygenation of 2-nitrobiaryls by triethyl phosphite it was shown that, in an analogous reaction of 2-o-nitrophenylpyridine (2.22), ring closure occurred at the electron-rich ring nitrogen atom rather than at



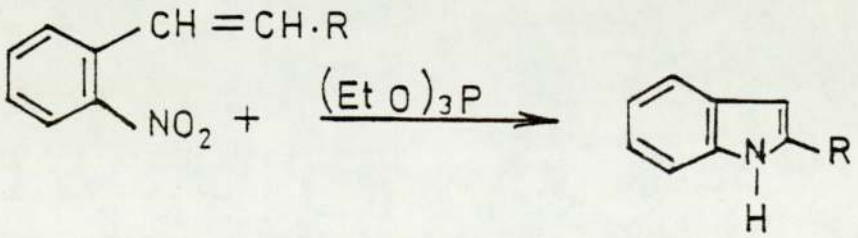
(2.22) (2.23)



(2.24) (2.25)



(2.26) (2.27)



(2.28) (2.29)

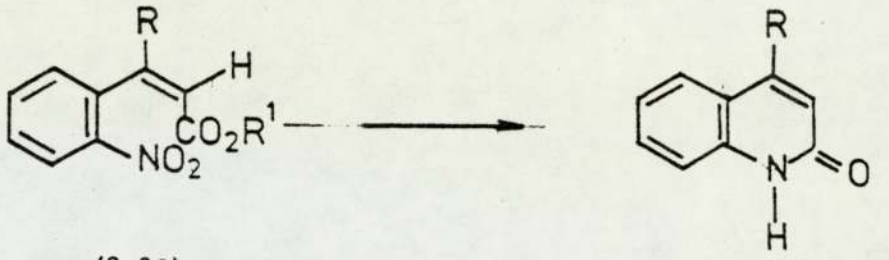
carbon to give the corresponding pyridoindazole (2.23) in a good yield.[102]

Comparable cyclisation of o-nitrobenzylidene-aniline (2.24) and o-nitroazoarenes (2.26) to 2-arylindazoles (2.25) and benzotriazoles (2.27) respectively[102,113] has been achieved.

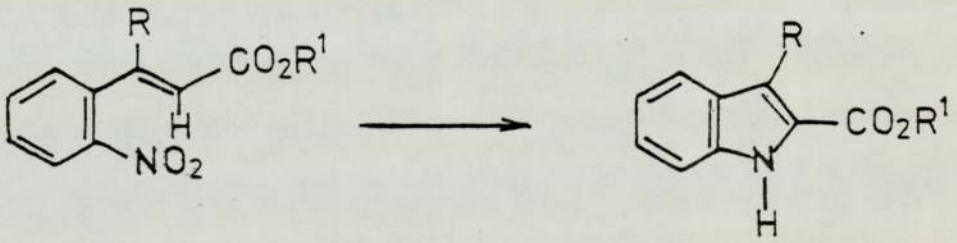
Extension of these reactions which have also been reported include the formation of pyrazolo[1,2-a]benzotriazoles from o-nitrophenylpyrazoles[114] and the synthesis of other related heterocycles.[115] Similarly, o-nitrochalcones (2.28; R = COAr) have been cyclised to 2-aroylindoles (2.29; R = COAr), which are difficult to obtain by other routes.[116]

Some interesting reactions involving the reductive cyclisation of a nitro group onto a carbonyl function using triethyl phosphite, have been noted, and the nature of the products is dependent on the stereochemistry of the starting material. For example the cis-o-nitrocinnamic acid ester (2.30) cyclises to yield the quinolone (2.31), while the trans (2.32) ester yields exclusively the indole (2.33).[117]

It has been reported that treatment of o-nitroalkylbenzenes[110,111] (2.34), with excess of boiling triethyl phosphite gives the corresponding triethyl N-alkylphosphorimidate (2.35) as the major identifiable product; in addition, minor amount of products ascribed to abstraction and insertion reactions of intermediate nitrenes were detected.[111] Thus o-propylnitrobenzene (2.34; R = n-pr) gave 2-methylindoline, o-propylaniline and o-allylaniline; o-butylnitrobenzene (2.34; R = n-but) gave 2-ethylindole, 1,2,3,4-tetrahydro-2-methylquinoline and unsaturated alkylanilines. These results are considered to confirm the intermediacy of a nitrene in the de-oxygenation, a conclusion supported in the considered judgement of Feuer.[106]



(2.30) (2.31)



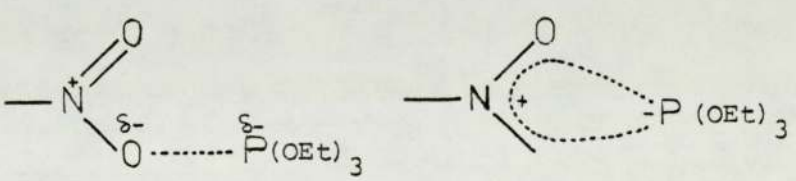
(2.32) (2.33)

(2.34) (2.35)

The foregoing pages demonstrate that trivalent phosphorous compounds will de-oxygenate nitro compounds. The temperature range to effect such reactions varies from 60°-155° depending on the nature of the substrate and the reactivity of the trivalent phosphorous reagent. It has not been found possible to arrest the reaction at an intermediate stage (scheme; 2.12 or 2.13) where the nitro group and trivalent phosphorous reagent are complexed in a 1 : 1 stoichiometric relationship. Such complexes have not been reported in the literature:reaction - if it occurs at all - proceeds to de-oxygenation and/or cyclisation.

However, the possibility exists that a trivalent phosphorous reagent could induce sufficient <u>polarisation</u> of the N-O bonds (Fig. 24) of the nitro group to influence the infra red spectrum. Such polarisation might be concentration dependent and provide a means of identifying nitro absorptions where they cannot be otherwise unambiguously assigned. Clearly the temperature at which such a process were to be conducted would be critical.

(Fig. 23) Possible nitro-phosphite polarisations



The postulated nitro-phosphite polarisations (Fig. 24) could assist in the spectral identification of the nitro group in complex molecules, for example, by causing a shift in the Ir spectrum. Shift reagents have been used widely in the simplification of complex spectra in nuclear magnetic resonance spectroscopy.[115,116]

As an example, the addition of cholesterol monohydrate to a solution of dipyridine adduct of trisdipivalomethanatoeuropium (III) ($Eu(DPM)_3$, HDPM represents dipivalomethane which is 2,2,6,6-tetramethylheptane-3,5-dione), both in carbon tetrachloride resulted in a substantial downfield shift in the proton resonance lines of the cholesterol from their normal positions.

Accordingly, a series of solutions was prepared consisting of N-methyl-o-nitrobenzamide in chloroform alone (Fig. 18) and solutions of the same amide in chloroform containing triethylphosphite in molar ratios ranging from 1:1 to 1:4. As can be observed from the spectra (Figs. 24-27) there is no change from the unperturbed spectrum of N-methyl-o-nitrobenzamides in chloroform alone either in the intensities of nitro absorptions, or in their frequencies. Furthermore when the solution containing N-methyl-o-nitrobenzamide and triethylphosphite in a molar ratio 1:4 was heated on a water bath at 70° for two hours there was again no change in the spectrum (Fig. 28).

It would appear therefore that the idea of using triethylphosphite as a nitro group 'shift' reagent is not applicable, at least in the o-nitrobenzamide series. o-Nitrobenzamides are resistant to de-oxygenating agents[117] and it is possible that the steric limitation imposed by the bulky ortho amide substituent prohibits intimate contact between the nitro group and the trivalent phosphorus reagent. It would be worth examining other nitro compounds (e.g. the corresponding m- and p-nitrobenzamides) to see if any perturbation of the nitro absorptions can be discerned. In addition the use of a more reactive trivalent phosphorus reagent could be examined.

Fig. 24

N-Methyl-o-nitrobenzamide
+ Triethyl phosphite (1:1)
in $CHCl_3$ at 25°

Fig. 25

N-Methyl-o-nitrobenzamide
+ Triethyl phosphite (1:2)
in $CHCl_3$ at 25°

Fig. 26

N . Methyl-o-nitrobenzamide
+ Triethyl phosphite (1:3)
in $CHCl_3$ at 25°

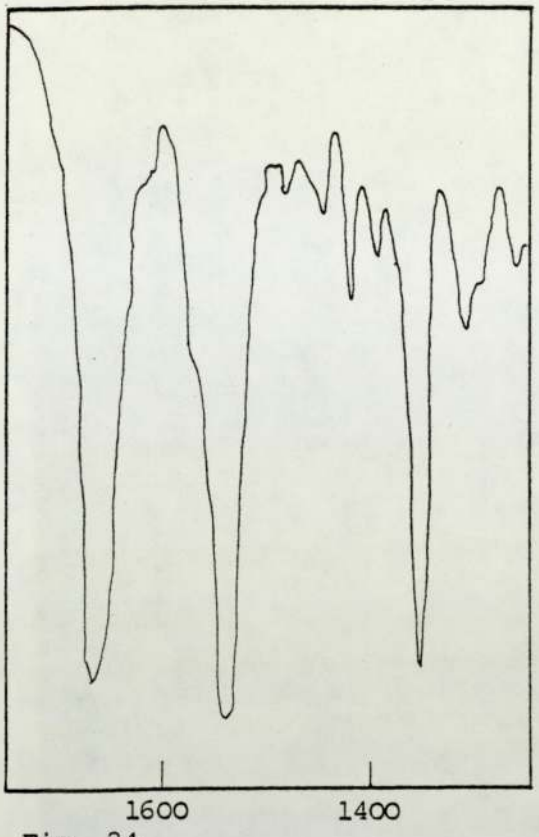


Fig. 24

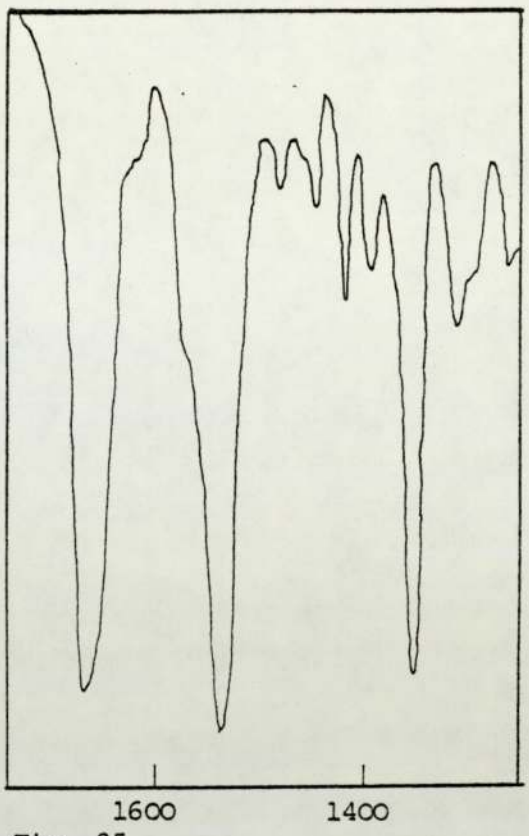


Fig. 25

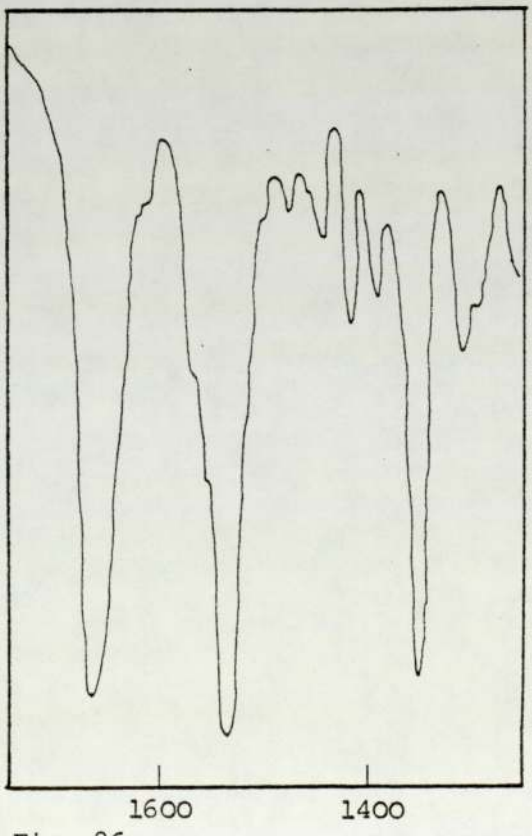


Fig. 26

Fig. 27

N-Methyl-o-nitrobenzamide
+ Triethyl phosphite (1:4)
in $CHCl_3$ at 25°

Fig. 28

N-Methyl-o-nitrobenzamide
+ Triethyl phosphite (1:4)
at 70°

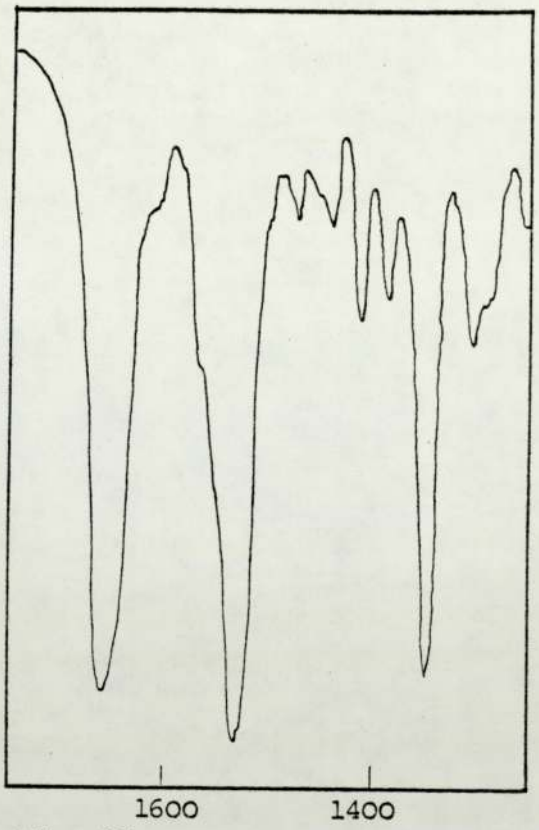


Fig. 27

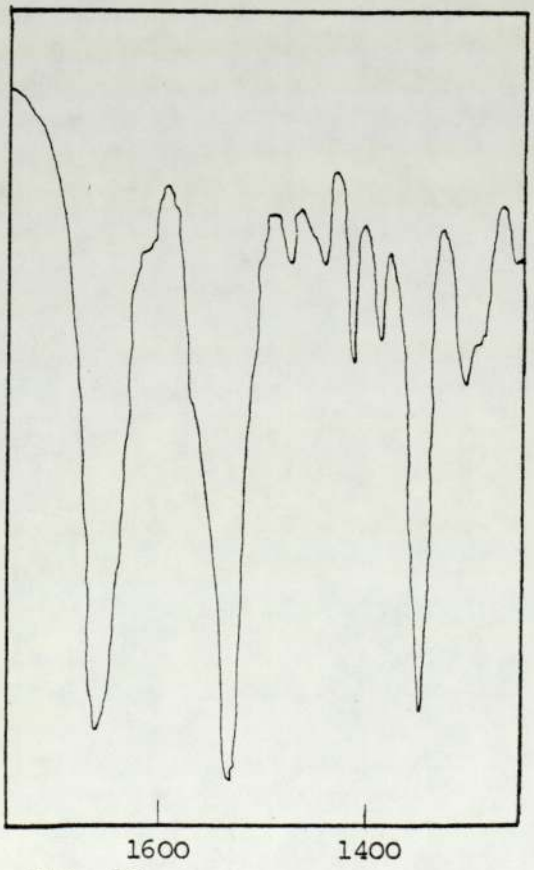


Fig. 28

CHAPTER III

PHOTOREARRANGEMENT OF N,N-DISUBSTITUTED 2-NITROBENZAMIDES

3.1 Introduction

The light-induced reactions of nitro compounds have been extensively studied. Recently, this category of reaction has been exposed to the wealth of separation techniques now available to the modern investigator. A range of novel structures has been identified in these photo-decompositions.

The range of photoproducts obtained by photolysis of aromatic nitro compounds depends on:

a) Solvent effects. For example,[121] irradiation of 2,6-dichloronitrobenzene (3.1) in propan-2-ol afforded the corresponding phenol (3.2) amine (3.3), hydroxylamine (3.4) and traces of nitrosobenzene derivatives (3.5), whereas irradiation in moist benzene afforded 2,6-dichlorophenol (3.2) as the sole product (scheme 9).

b) Sensitiser or quencher effects. Sensitisation occurs in a donor-acceptor system when only the donor absorbs the incident light, and the triplet energy of the donor is much greater than the triplet energy of the acceptor (at least 3 KCal/mol). Light absorption by the donor produces singlet excited donor (^1D) which undergoes intersystem crossing, giving triplet excited donor (^3D). Triplet excited donor then collides with acceptor, producing triplet excited acceptor (^3A) and ground state donor (D).

$$D + h\nu \longrightarrow {}^1D$$

$${}^1D \xrightarrow[\text{crossing}]{\text{intersystem}} {}^3D$$

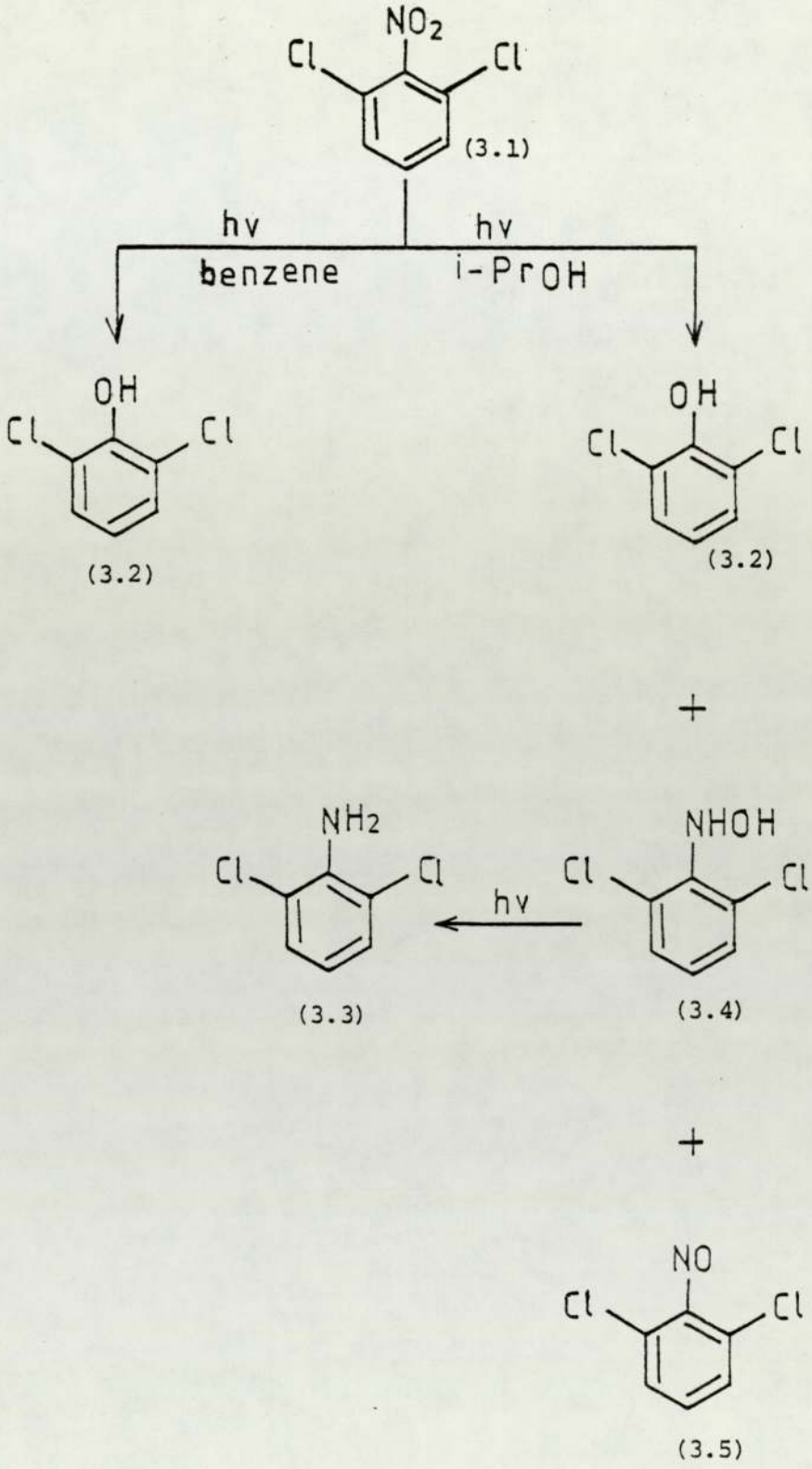
$${}^3D + A \longrightarrow D + {}^3A$$

$${}^3A = \text{product (sensitization)}$$

$${}^3D = \text{product (quenching)}$$

The concentration of the acceptor must be kept low enough to make

(scheme 9)


NO2
Cl
Cl
(3.1)
hv
benzene
hv
i-PrOH
OH
Cl
Cl
(3.2)
OH
Cl
Cl
(3.2)
+
NH2
Cl
Cl
hv
NHOH
Cl
Cl
(3.3)
(3.4)
+
NO
Cl
Cl
(3.5)

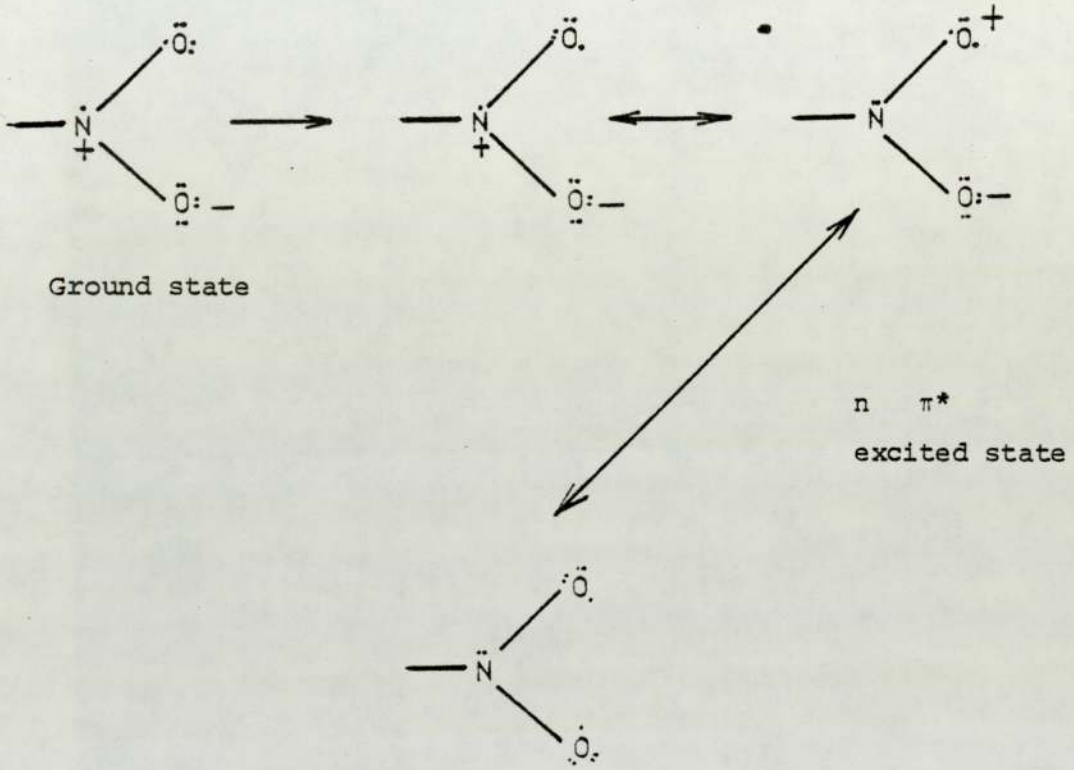
collisions with singlet excited donor improbable. This concentration will be determined by the singlet lifetime of the donor. If (^{3}A) gives the products of interest, this is a called sensitization mechanism. If the product of interest are derived from (^{3}D), (A) is a quencher and this is a quenching mechanism. Sensitization and quenching are important methods for determining the spin multiplicity of excited states responsible for photochemical reactions.[122] Sensitization is also an important method for producing the triplet states of molecules in which the efficiency of intersystem crossing is low. The chemistry of singlet and triplet excited states is often quite different. Photochemical reaction is commonly completed <u>via</u> the lowest lying singlet and triplet excited states of the substrate which are usually more stable (scheme 10 in $-NO_2$ group case). An example[123] of the quenching effect in the photochemistry of nitro compound is given below. The presence of 1,3-pentadiene and/or oxygen which acts as triplet quencher, in the photoreduction of 4-nitropyridine considerably lengthens the reaction time. On the other hand benzophenone acts as sensitizer and accelerates the reaction.

c) The wavelength range of the light source in use. Short wavelength Uv light leads predominantly to complete photoreduction of nitrobenzene to aniline, whereas Uv (λ 290 mμ) induces the formation of bimolecular species (e.g. azo and azoxy compounds) as major products.[124]

d) Nature and position of the substituents on the aromatic ring in relation to the nitro group.

In the present work, the photochemistry of 2-nitrobenzanilides has been examined.

(scheme 10)

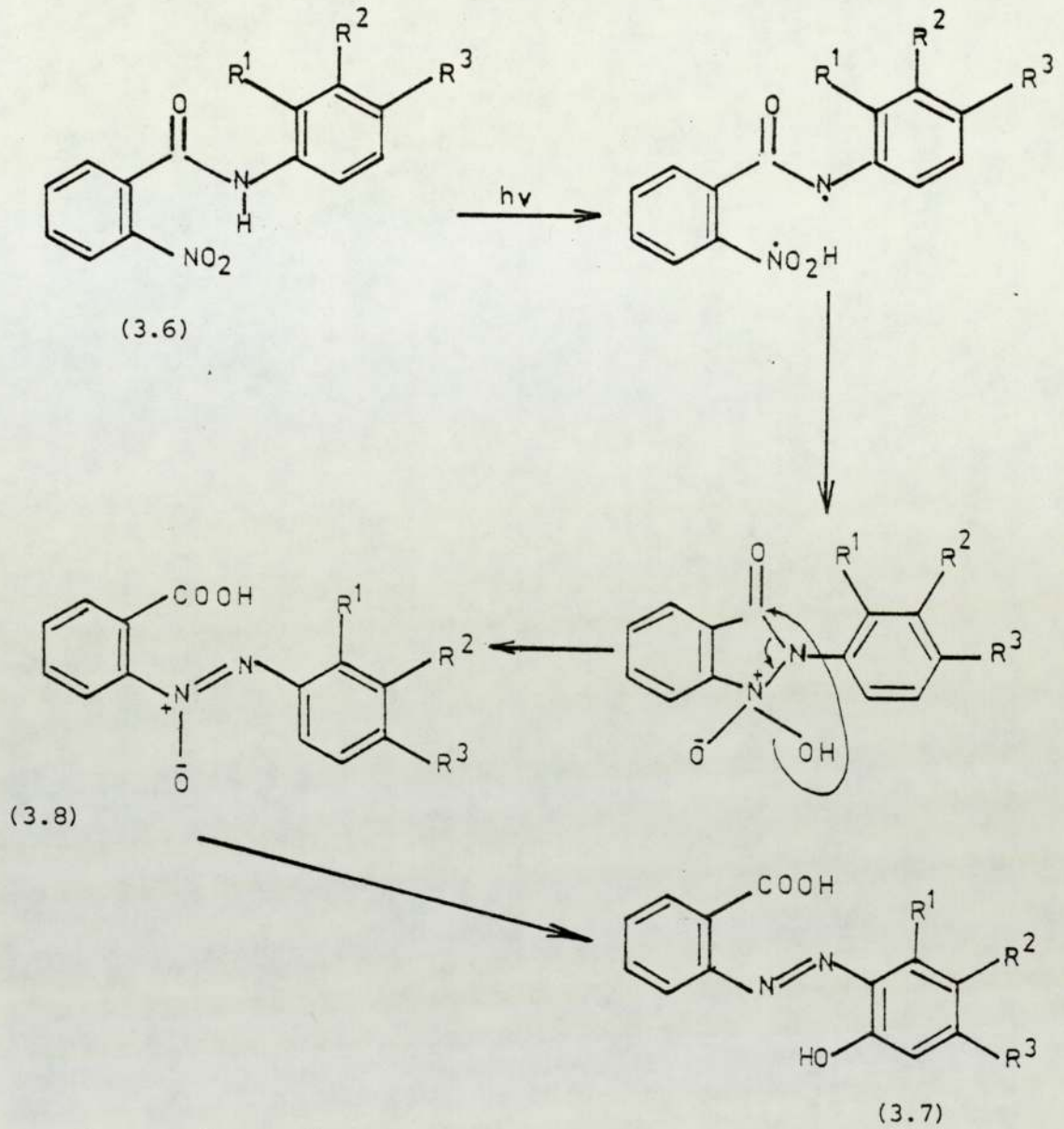


Ground state

n π*

excited state

(scheme 11)



(3.6)

(3.8)

(3.7)

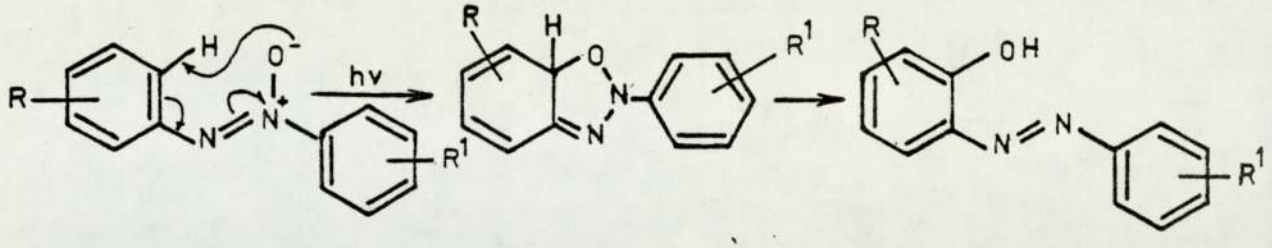
	R^1	R^2	R^3
a	H	H	H
b	Cl	H	H
c	H	H	Cl
d	Me	H	H
e	H	H	Me
f	-CH:CH.CH:CH-		H
g	NO_2	H	H
h	H	H	NO_2
i	CN	H	H
j	H	H	CN

Gunn and Stevens[125] observed that irradiation of the cream-coloured ethanol solution of o-nitrobenzanilide (3.6a) leads to a dark-red solution from which 15% of the azohydroxycarboxylic acid (3.7a) could be isolated. Full details of the photorearrangement of a series of N-aryl-o-nitrobenzanilides (3.6) to 2-(2-hydroxy-phenylazo)benzoic acids (3.7) have been published. This photoisomerisation appears to be general property of N-aryl-o-nitro-benzamides but not the N-alkyl analogues. The chloroanilides (3.6b and c) and the o- and p-tolyl isomers (3.6d and e) are even more photo-sensitive than unsubstituted 2-nitrobenzanilide. The photo-products were assigned structures (3.7b - e) respectively. Also the 2-nitrobenzoyl derivative of 1-naphthylamine (3.6f) afforded the photoisomer (3.7f). In contrast it has been noted that anilides bearing electron-attracting substituents (3.6g - j) were stable in the crystal phase over a long period and were not subject to solution photolysis (scheme 11).

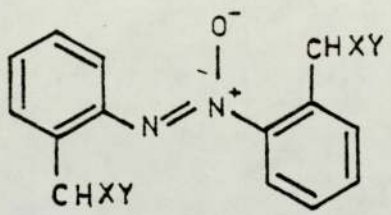
The intermediacy of azoxybenzene-2-carboxylic acids (3.8) in these rearrangements has been confirmed and their formation rationalized in terms of an initial hydrogen abstration (scheme 12). Further photolysis of the intermediate (3.8) proceeds by migration of the azoxy oxygen atom to the ortho- position of the more distant aromatic nucleus to give (3.7).

In agreement, Badger and Buttery[126] found that unsymmetrical azoxybenzenes such as (3.9)rearrange exclusively to (3.11), where the azoxy oxygen migrates to the aromatic ring farther from its point of departure. They accordingly proposed a cyclic intramolecular arrangement initiated by nucleophilic attack of the azoxy oxygen on the appropriate ring carbon, giving a cyclic intermediate (3.10).

(scheme 12)

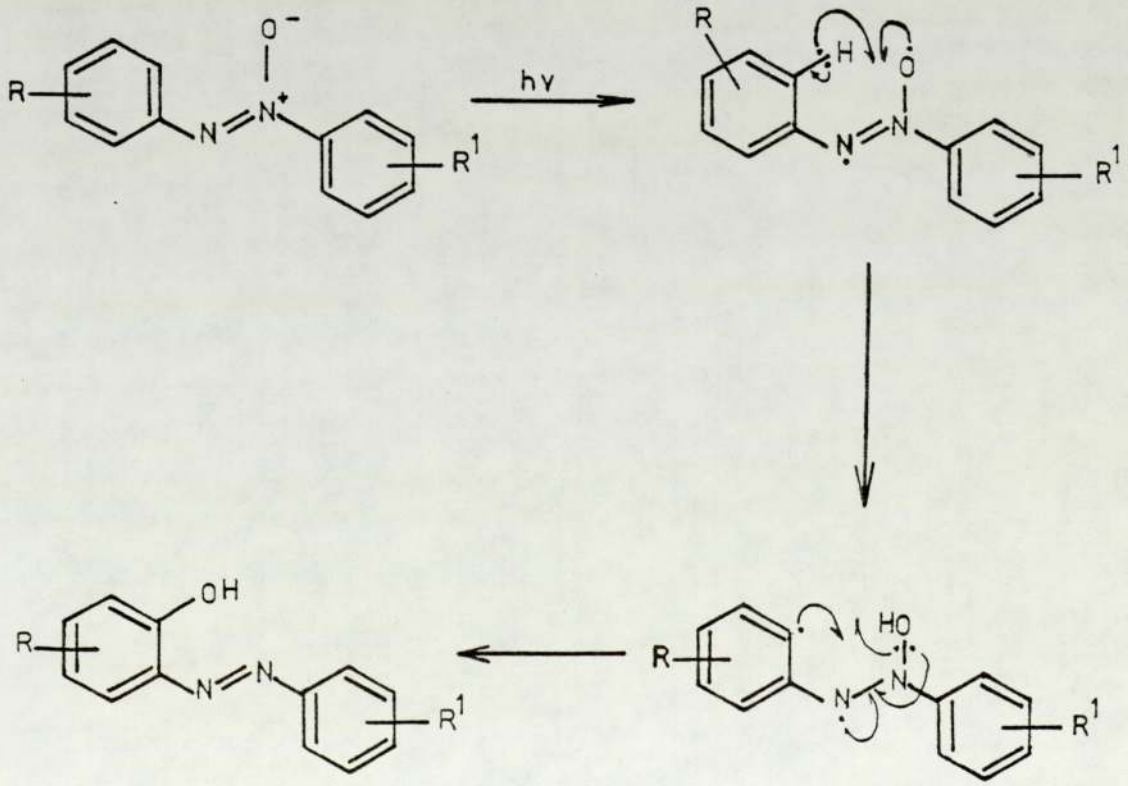


(3.9) (3.10) (3.11)



(3.12)

(shceme 13)

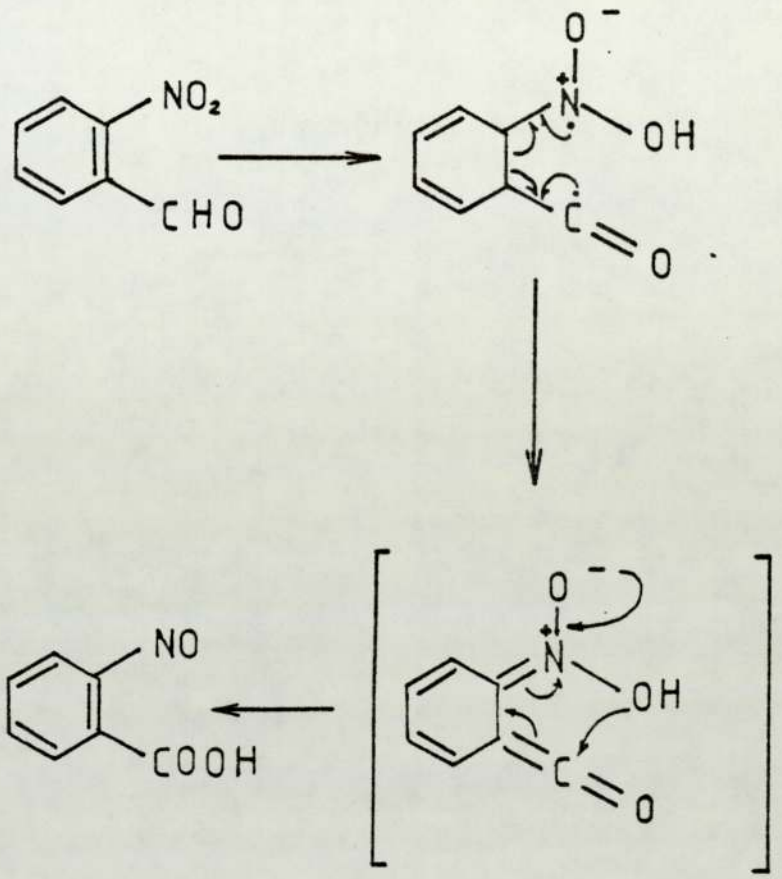


The carbon ortho to the azoxy linkage in this case is activated to nucleophilic attack by the electron-attracting phenylazoxy-substituent. This proposal has been confirmed[126] by isotopic labelling experiments. Oae et al [127] in contrast suggested a radical mechanism for this step in which homolytic hydrogen abstraction from the conveniently situated ortho position of the distant aromatic ring is followed by hydroxyl transfer (scheme 13). (Note the similarity to the widely accepted photo-rearrangement of 2-nitro benzaldehyde to 2-nitrosobenzoic acid (scheme14.)

Bunce et al [128] have carried out a series of experiments to further investigate the azoxybenzene ⟶ 2-hydroxyazobenzene photo-rearrangement in an attempt to distinguish between ionic and free radical mechanisms. They deduced strong evidence against a hydrogen abstraction mechanism for the azoxybenzene photo-rearrangement. Firstly, the benzylic C-H bond of the 2,2'-disubstituted azoxybenzenes (3.12) are unreactive despite the lower bond dissociation energies of benzylic compared with aryl C-H bonds. Secondly, the rate of rearrangement increases in polar solvents, suggestive of a polar rather than a radical pathway. Thirdly, the deuterium isotope effect is far too small to be associated with a primary isotope effect for abstraction of the strongly bound aryl hydrogen. They added that a $\pi \longrightarrow \pi^*$ state is responsible for the azoxybenzene photo-rearrangement, whereas most photochemical hydrogen abstractions proceed from $n \longrightarrow \pi^*$ states. Although exceptions are known, this behaviour is suggestive of a mechanism not involving hydrogen abstraction.

Recently, Bunce et al [129] have published more evidence supporting the intermediacy of the previously mentioned intra-molecular cyclic intermediate (3.10). They reported that the

(scheme 14)

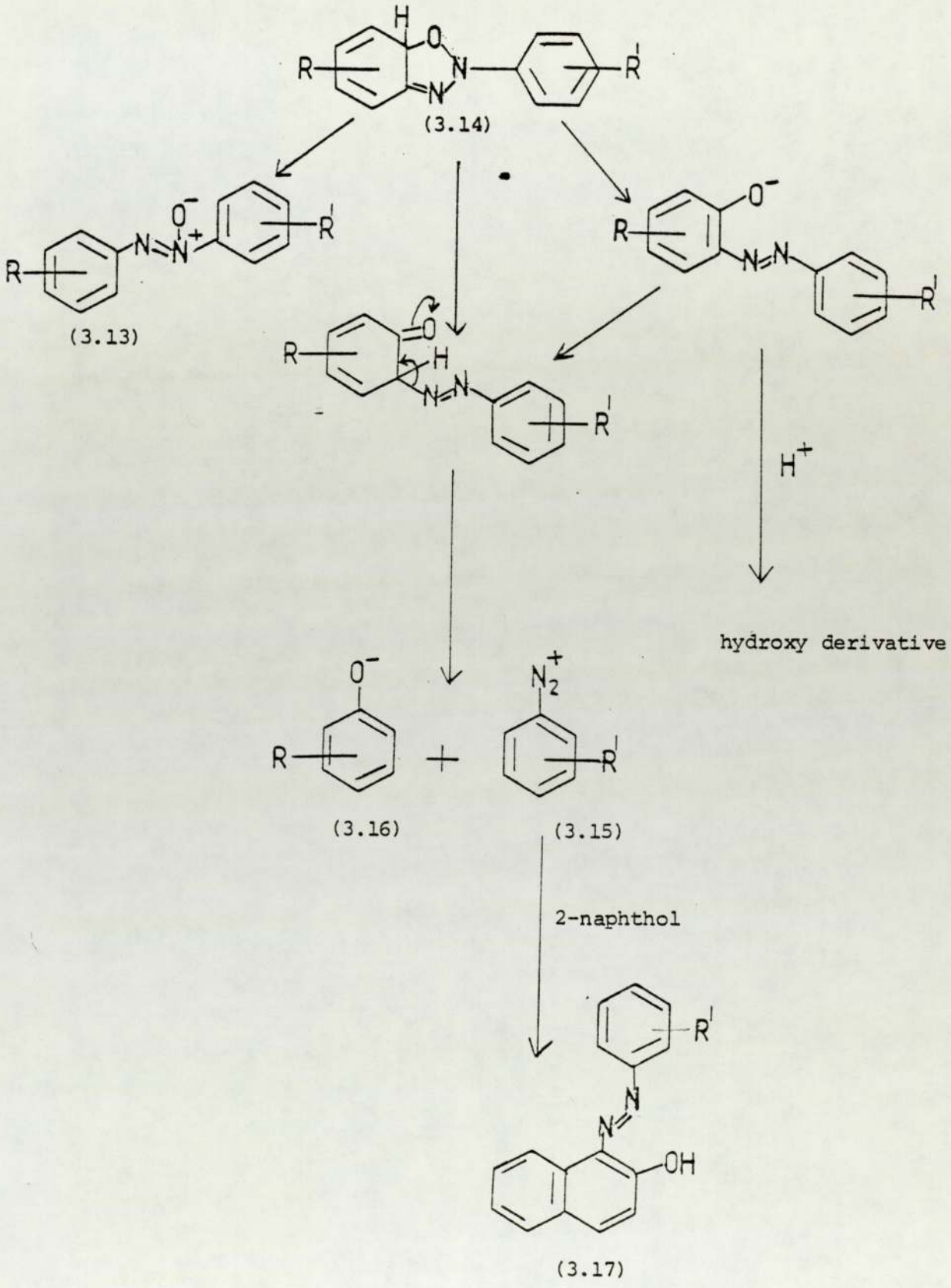


excited state responsible for the azoxybenzene photo-rearrangement is $n \longrightarrow \pi^*$, not $\pi \longrightarrow \pi^*$ as assumed previously. They found a good agreement between the calculated and observed spectral location of the excited states bands ($\pi \longrightarrow \pi^*$ and $n \longrightarrow \pi^*$). The theoretical model predicts an electrophilic role for oxygen during the rearrangement. Experiments with substituted azoxybenzenes support this model. They noted that among isomeric compounds having different substituents on the two rings, a higher quantum yield of reaction is observed when the oxygen is to migrate into a ring bearing a more electron-donating substituent. Bunce[129] also reported the involvement of an aryldiazonium ion in the photolysis of azoxybenzenes in nonbasic solvents which leads quite generally to azonaphthol-derivatives in the presence of 2-naphthol. He postulated a free diazonium ion as a precursor of the azonaphthol (3.17) as shown in (scheme 15). Bunce[130] noted that, although no direct observation of diazonium ions has been made, it is consistent with their involvement in the photolysis of p-substituted azoxybenzenes in benzene 2-naphthol. He added that it was possible that the naphthol, rather than trapping a free cation attacks some intermediate such as (3.14) and abstract the diazonium moiety (3.15) with expulsion of the phenol or phenoxide (3.16) (scheme 14). He observed that variation of the solvent, light intensity, and substrate concentration all cause a change in the product distribution.

The next section of this thesis will examine the photo-chemistry of N-alkyl-o-nitrobenzanilides (3.18).

Also in an attempt to generalise the case of photolysis of ortho-substituted nitro compounds, the light-induced reaction of N-(o-nitrobenzoyl)tetrahydroquinoline (3.23) has been examined.

(scheme 15)



All the starting materials required in the course of this work were prepared from 2-nitrobenzoyl chloride and the appropriate amine by the published routes.[131,132]

3.2 Photolysis of N-Ethyl-o-nitrobenzanilide

As has been reported [125] in the photolysis of o-nitrobenzanilides (3.6), the primary photo-process was assumed to involve initial hydrogen atom abstraction from the amidic nitrogen. Accordingly it was anticipated that if this amidic nitrogen were alkylated, the compound should be photostable.

Surprisingly, N-ethyl-o-nitrobenzanilide (3.18a) was found to be extremely light sensitive both in the solid state and in 95% ethanol solution.

When dry pure crystals of N-ethyl-o-nitrobenzanilide (3.18a) were exposed to sunlight for several weeks, a brown colour developed on the crystal surfaces; also when a solution of the compound in 95% ethanol was irradiated with an unfiltered 100 W medium pressure lamp, it rapidly turned bright yellow, changing to dark red in only a few hours. The yield of coloured products reached a maximum after about 12 hours. Irradiation of a dilute solution in a quartz cuvette was used to follow the progress of the reaction by uv-visible spectroscopy. Coversion reached a maximum after two hours.

After 48 hours the examination of the photolysate (5 g of (3.18a) in 1000 mls of 95% ethanol) was carried on silica gel (0.25 mm) using two different solvents. The use of ethyl acetate-methanol-ammonia (4:4:1) as developing solvent clearly revealed the presence of two red products having Rf values 0.4 and 0.5 as the major products, as well as substantial amounts of unreacted starting material (Rf value 0.87). With the use of chloroform-methanol (9:1), the chromatogram showed the presence of at least ten other products, the highly coloured ones having Rf values 0.02,

0.06, 0.14, 0.27, 0.37 and 0.41 (Fig. 29).

Separation of the red photo-products

The red products were isolated by exploiting their acidic character and their property by being tenaciously adsorbed on a neutral alumina chromatography column. The acidic compounds were desorbed from the extruded column with 2N-sodium hydroxide solution and reprecipitated with hydrochloric acid. The red compounds were extracted into absolute alcohol. Chromatographic fractionation on a silica gel column resulted in the separation of two red compounds. The main product crystallised as bright red-orange needles from aqueous ethanol (mp 87 - 90°); and the minor product formed maroon plates from benzene (mp 203 - 205°).

Combined yields of these compounds on repeated photolyses were of the order of 10 - 15%; the inefficient conversion was presumably due to competitive light adsorption by the coloured photo-products. The efficiency of the photolysis could be improved by continuously circulating the photolysate through a neutral alumina column by means of peristalsis pump (Fig. 30). In this way unreacted starting material was continuously returned to the reaction vessel by elution with the circulating ethanol and the red photoproducts remained adsorbed on the column. The efficiency of the photolysis was thus improved to give 15 - 20% combined yields of the red products.

Identification of the red compounds

The two red photo-products isolated from the photolysis mixture of N-ethyl-o-nitro-benzanilide (3.18a), were identified spectroscopically as azobenzene-2-carboxylic acid (3.19) and

Fig. 29. TLC of the Photolysate of N-Ethyl-o-nitrobenzanilide

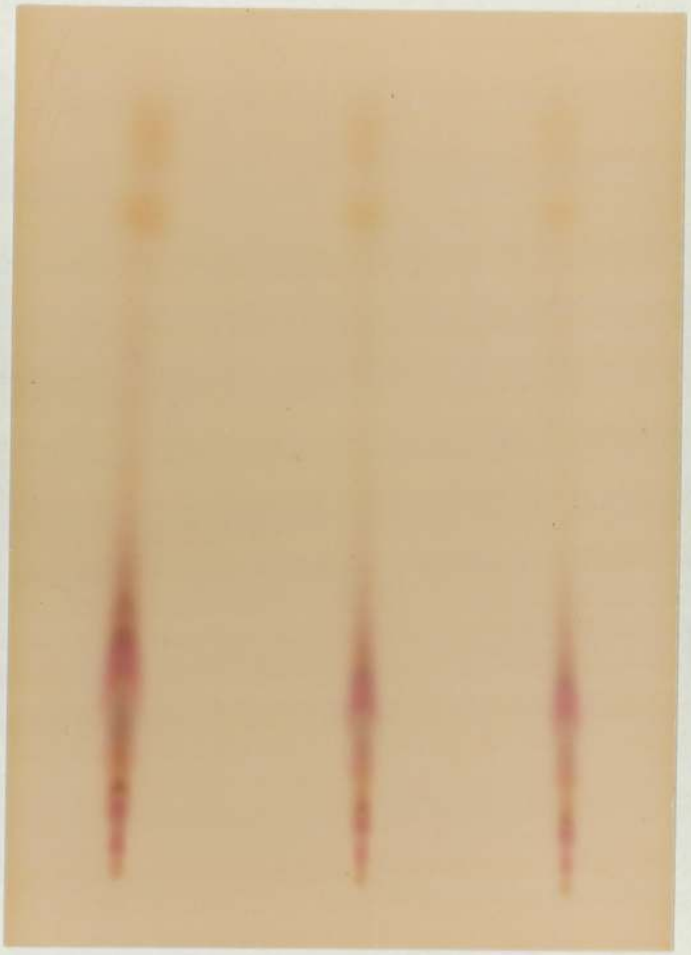
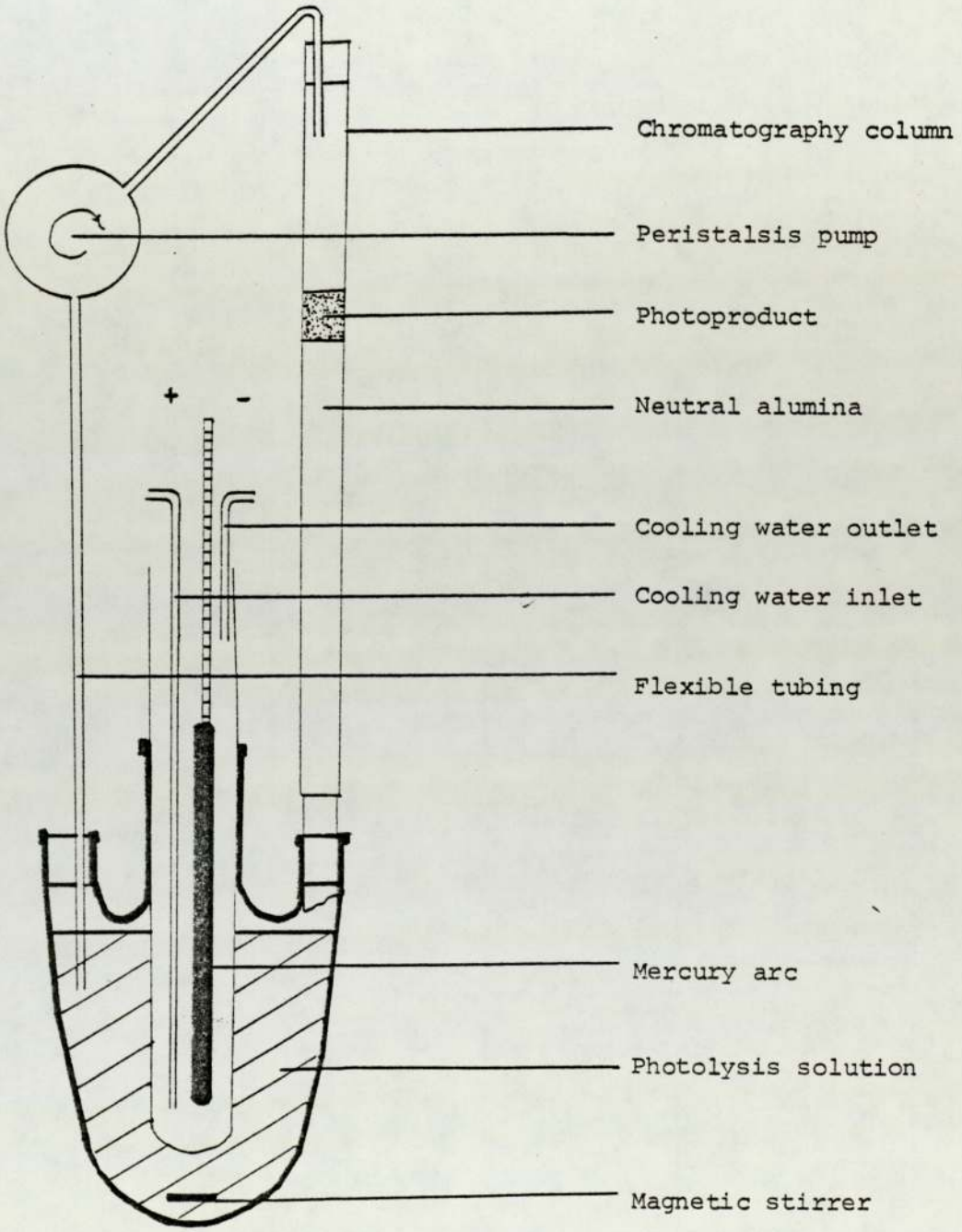
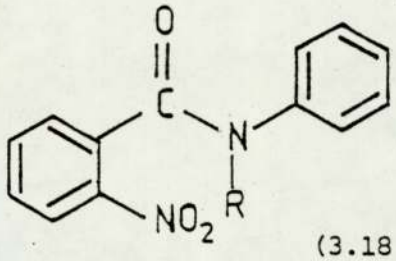


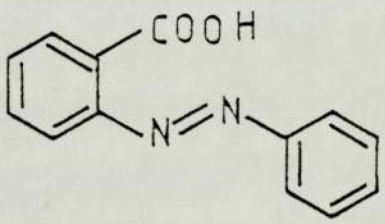
Fig. 30 Hanovia 100 watt medium pressure 1 litre Photochemical Reactor





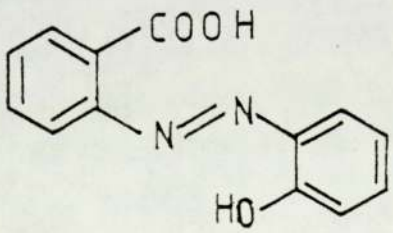
(3.18)

hv 95% Ethanol



(3.19)

+



(3.20)

a: R = Et

b: R = Me

2'-hydroxyazobenzene-2-carboxylic acid (3.20).

The electronic absorption spectra of compounds (3.19) and (3.20) showed long wavelength absorptions at 317 nm and 325 nm respectively (Fig. 31).

The ir spectra of the azobenzoic acid derivatives (3.19) and (3.20) show two medium strength bands at 1440 cm^{-1} and 1410 cm^{-1} due to the -N=N- stretching frequency. The spectra also show strong bands at 1720 cm^{-1} and 1680 cm^{-1} associated with carbonyl stretching absorptions for both (3.19) and (3.20) respectively, and a characteristic band for the bonded OH group at 3200 - 2300 cm^{-1} for compound (3.20).

The ion-impact promoted fragmentations of (3.19) and (3.20) isolated in the present work, correspond closely to those reported for other azo compounds.[125] (Fig. 32) shows the plot of relative abundance (%) versus m/e values from the mass spectrum of the azobenzene-2-carboxylic acid (3.19) separated from the photolysis of (3.18a). The dominating process in both compounds is cleavage of the C-N bonds (scheme 16) to give diazonium ions which are further fragmented by loss of nitrogen to give the respective aryl cations. The mass spectra both show abundant ions at m/e 149, 121, 93 and 65 (Route A); also in the case of compound (3.19) at m/e 105 and m/e 77 formed by cleavage of the alternative C-N bond by route B.

The mass spectral fragmentation of the hydroxyazobenzene (3.20) showed the same pattern of cleavage but the differing abundances of the various ions (Fig. 33) presumably reflects the influence of the hydroxy group, which, by conjugation with the diazonium group (3.21 ⟷ 3.22) confers double-bond character, and

Fig. 31 Uv spectra of azobenzene-2-carboxylic acid and 2'-hydroxyazobenzene-2-carboxylic acid in 95% ethanol

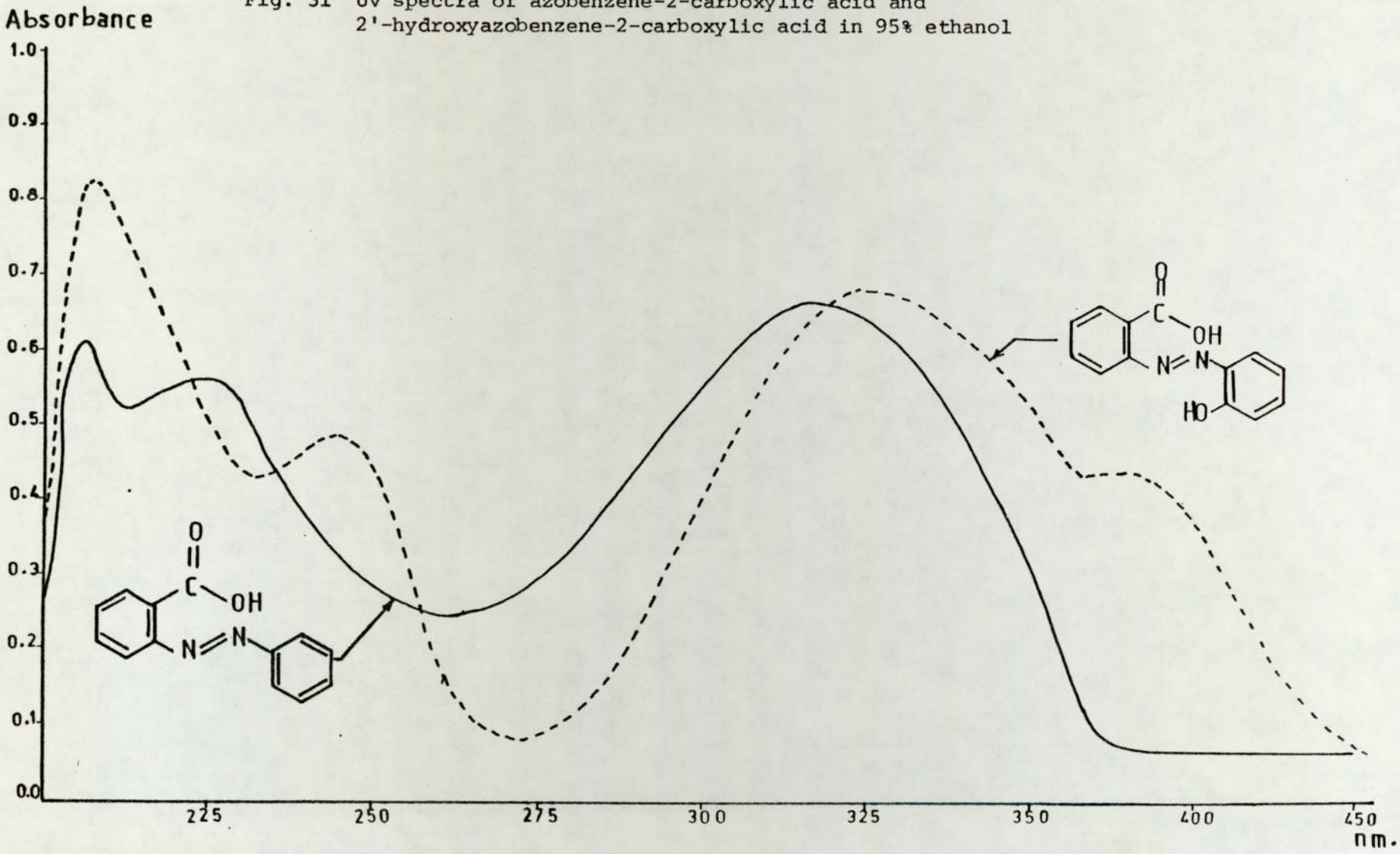


Fig. 32 Mass spectrum of azobenzene-2-carboxylic acid

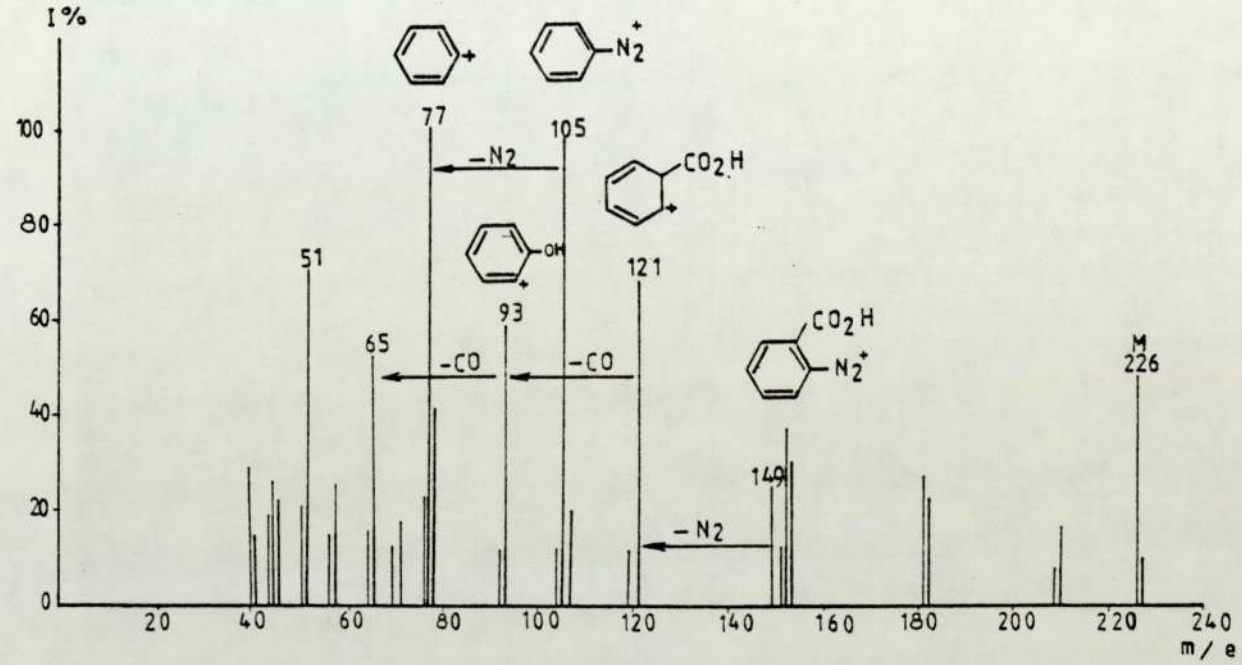
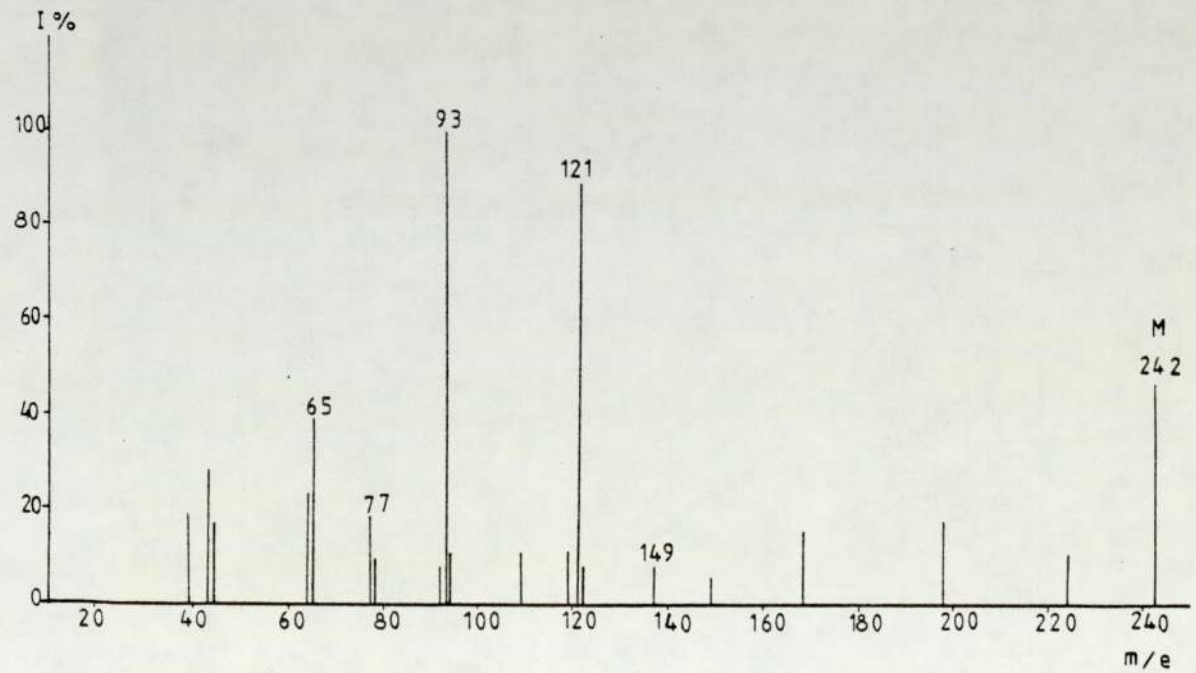


Fig. 33 Mass spectrum of 2'-hydroxyazobenzene-2-carboxylic acid



hence stability, on the bond linking the diazonium group with the hydroxyaryl moiety (scheme 16).

On catalytic reduction followed by tlc identification (3.19) was reduced to aniline and anthranilic acid as expected. Also the compound proved to be identical to the orange compound prepared by condensation of 2-nitrosobenzoic acid and aniline in acetic acid at room temperature and reported in the literature as azobenzene-2-carboxylic acid.[133]

All the previous positive tests confirmed the structures of the isolated red products from the photolysis of N-ethyl-o-nitrobenzanilide (3.18a) to the azobenzene-2-carboxylic acid (3.19) and 2'-hydroxyazobenzene-2-carboxylic acid (3.20).

Identification of other photolytic products was attempted by comparing tlc chromatograms of the photolysate with appropriate reference samples (Table 17). Three compounds were positively identified : 2-nitrosobenzoic acid, aniline, and N-ethylaniline. Other products (see table 17) were conclusively shown not to be present, and several of the coloured products formed in the photolysis of N-ethyl-o-nitrobenzanilide remain to be identified.

(scheme 16)

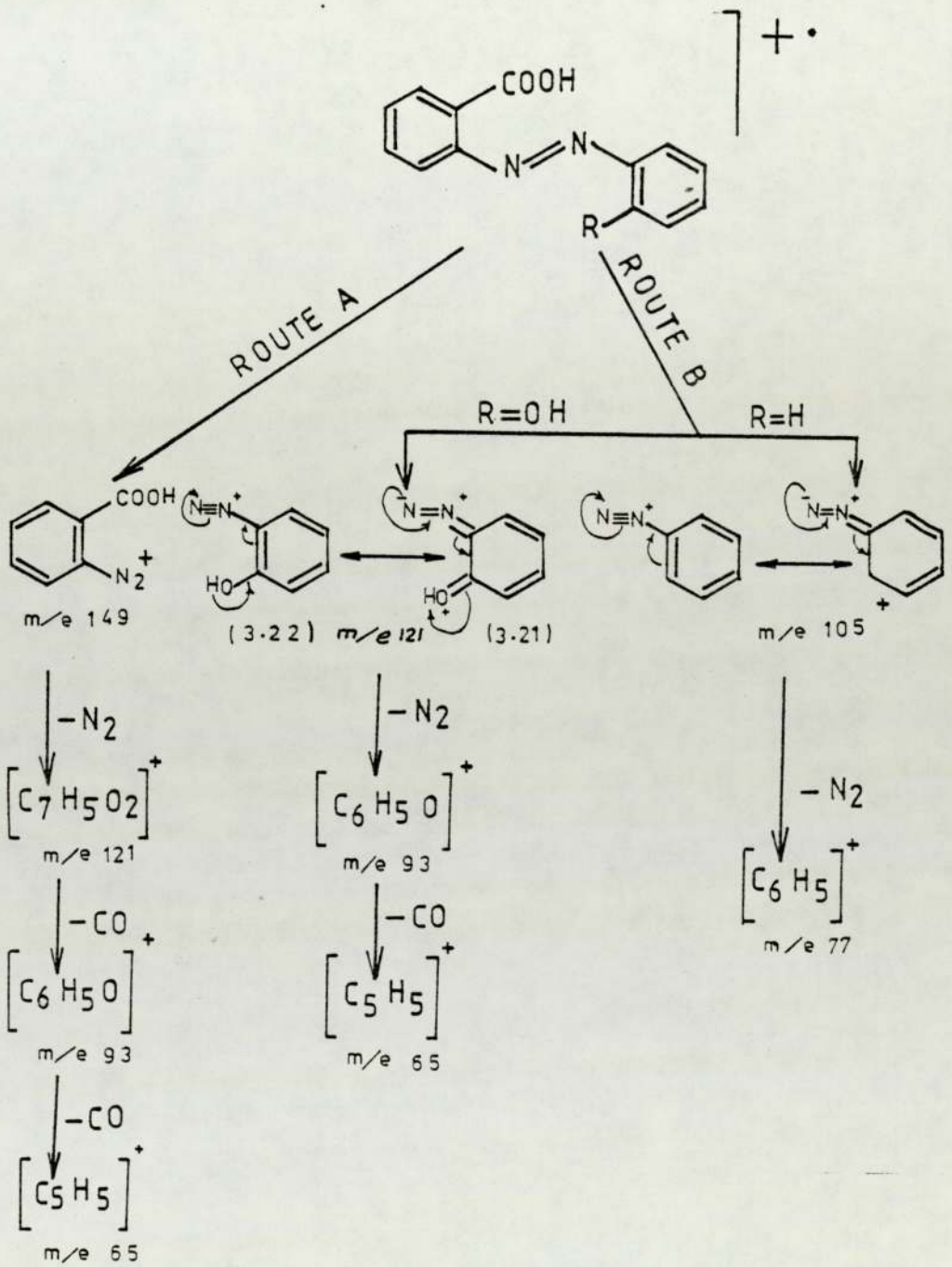

COOH
N=N
R
ROUTE A
ROUTE B
R=OH
R=H
m/e 149
(3.22)
m/e 121
(3.21)
m/e 105
-N2
-CO
m/e 93
m/e 65
m/e 77

Table 17 Comparison on TLC between some Photolysis Products and Prepared Reference Samples

Compound Reference	Observation on tlc (3.18a)	(3.18b)
Indigo	-ve	-ve
2-Methyl -3-phenylquinazol-4(3H) -one	-ve	-ve
o-Nitrosobenzoic acid	+ve	+ve
Anthranilic acid	-ve	-ve
o-Nitrobenzanilide	-ve	-ve
Aniline	+ve	+ve
N-Alkylaniline	+ve (alkyl= Et)	+ve (alkyl= Me)
Anil*	-ve	-ve
Isatogen	-ve	-ve

* Ethylidineaniline in the case of (3.18a) and methylidineaniline in the case of (3.18b)

3.3 Irradiation of N-Methyl-o-nitrobenzanilide

Photolysis of N-methyl-o-nitrobenzanilide (3.18b) was expected to follow the same pattern as the ethyl analogue (3.18a).

Irradiation of (3.18b) in 95% ethanol resulted in the isolation of (3.19) and (3.20) but in better yield than in the photolysis of (3.18a) (25% combined yield after circulation using a peristalsis pump, Fig. 30).

Spectral comparisons confirmed the identification of the same two red products (Fig. 31 shows the uv spectrum and Fig. 32, 33 and scheme 16 for MS); also the melting points are consistent with the products previously separated from (3.18a).

Therefore the photolysis of N-methyl-o-nitrobenzanilide (3.18b) like the N-ethyl analogue afforded azobenzene-2-carboxylic acid (3.19) as the major photolytic product and 2'-hydroxy-azobenzene-2-carboxylic acid as a minor identifiable photolytic product.

Beside these two compounds, tlc examination revealed the presence of the starting material, N-methylaniline, aniline and o-nitrosobenzoic acid as well as several highly-coloured but unidentified products found among the photolysate of N-ethyl-o-nitrobenzanilide (3.18a).

Tlc also confirmed the absence of the anil, anthranilic acid, isatogen, o-nitrobenzanilide and indigo (Table 17).

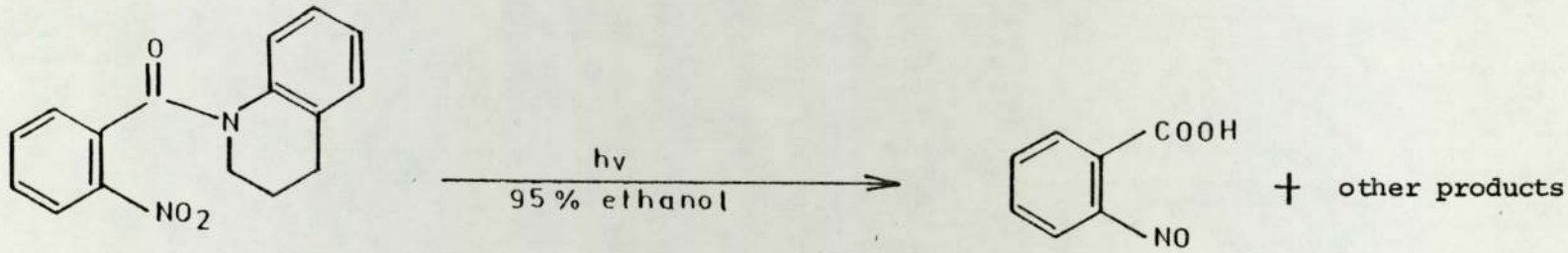
Generally speaking, therefore, there is no difference between the photolysis of N-ethyl and N-methyl-o-nitrobenzanilides.

3.4 Irradiation of N-(o-nitrobenzoyl)-1,2,3,4,-tetrahydroquinoline

To complete the series of photo-investigations of N-substituted-o-nitrobenzanilide derivatives, N-(o-nitrobenzoyl)-1,2,3,4-tetrahydroquinoline (3.23) was examined. This is an interesting benzanilide derivative in that the N-alkyl group is fused back to the N-aryl ring.

N-(o-Nitrobenzoyl)-1,2,3,4-tetrahydroquinoline (3.23) was found to be light sensitive in both solid and solution phases. Dry crystals of the quinoline turn brown when exposed to sunlight for a few weeks. After three hours irradiation in 95% ethanol the solution turns a lime green colour. After 48 hours tlc examination of the photolysate (0.3% in 95% ethanol), on silica gel_{254} (0.25 mm, chloroform-methanol 9:1) established the presence of about twenty new photolytic products most of them fluorescent under uv light (254 nm), together with an appreciable amount of the starting material (Rf = 0.9). Apparently no red azo-compounds were formed as had been noted in the foregoing N-alkyl derivatives.

When the photolysate was eluted through a neutral alumina chromatography column a dark green band was adsorbed on the top of the column. The green layer was desorbed from the extruded column with an alkali (2N-NaOH) reprecipitated again with an acid (conc HCl), and filtered. The precipitate was extracted with alcohol, then the solvent evaporated to leave very fine crystals on the wall of the flask (whitish green crystals from ethanol, m.p. 207). This compound was the major photolytic product (about 20% after circulation through alumina column by means of peristalsis pump (Fig. 3Q)).



(3.23)

(3.24)

The major photolytic product was (3.24) identified spectroscopically and found to be identical with a sample of o-nitrosobenzoic acid prepared independently.

Spectral identification of the isolated o-nitrosobenzoic acid

The mass spectrum of o-nitrosobenzoic acid (Fig. 34) is strikingly similar to that of o-nitrobenzaldehyde (Fig. 35).[134] The aldehyde shows a base peak (m/e 121) due to loss of NO: the formation of a C-O bond during fragmentation is indicated by the subsequent decomposition of the M-NO ion (m/e 121) through successive losses of carbon monoxide, finally affording m/e 65 $\left[C_5H_5\right]^+$, which contains all of the hydrogens present in the original molecule but none of the heteroatoms. Since the photolytic conversion of o-nitrobenzaldehyde to o-nitrosobenzoic acid has long been known,[135] the possibility arises that a similar isomerization might occur upon electron impact,[136] thus accounting for the unusually facile loss of nitric oxide. In fact o-nitrosobenzoic acid decomposes by exactly the same sequence (successive loss of nitric oxide, carbon monoxide, and carbon monoxide again) necessitating oxygen (probably hydroxyl) migration at some stage in the decomposition (scheme 17). However, the relative abundances of the pertinent ions in the spectra of (3.24) and (3.25) are very different: unlike o-nitro-benzaldehyde the isomeric nitrosobenzoic acid shows a large molecular ion. Decomposition of the two substances via a common molecular ion therefore seems unlikely. Hence there is no evidence at present to suggest that the facile loss of nitric oxide from o-nitro-benzaldehyde proceeds other than by the usual isomerization to nitrite, perhaps facilitated by the o-aldehyde group.

Fig. 34. Main spectrum of o-nitrosobenzoic acid

(scheme 17)

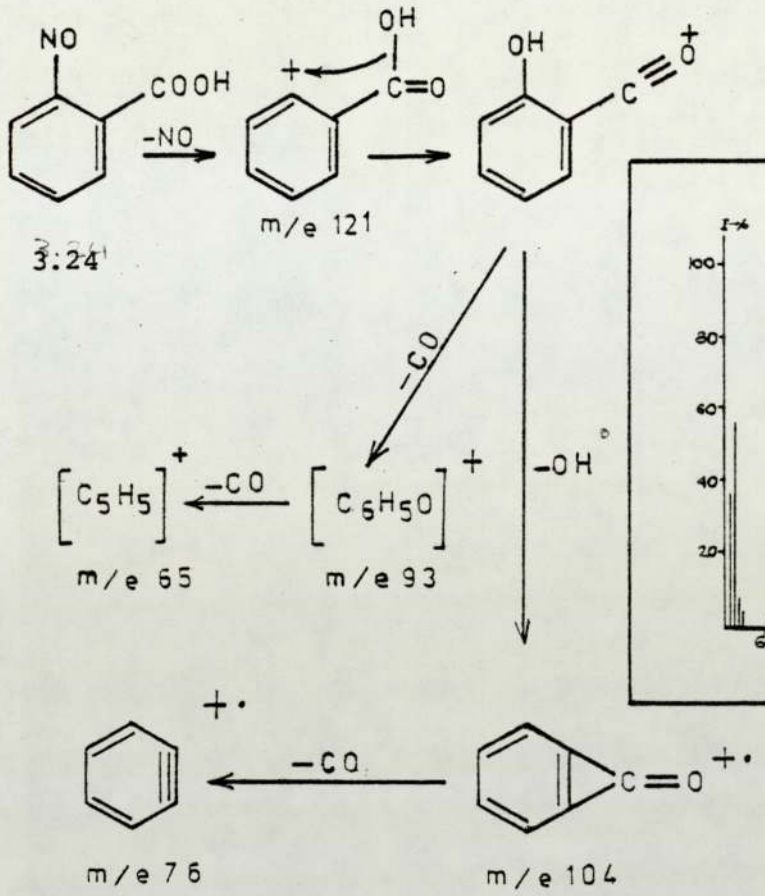


Fig. 35

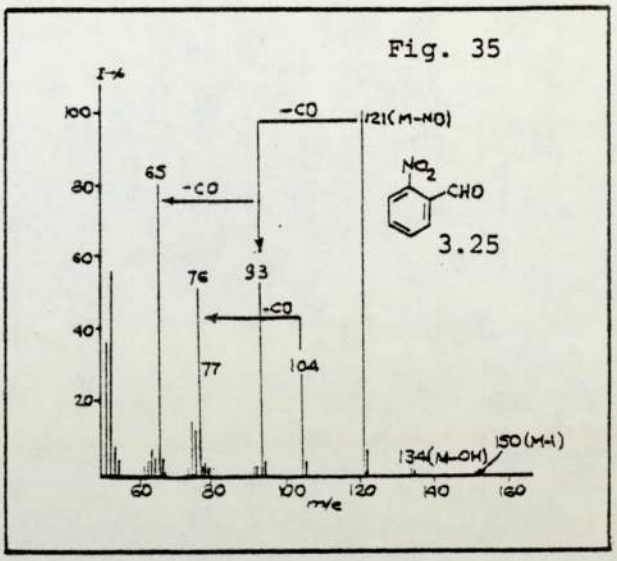
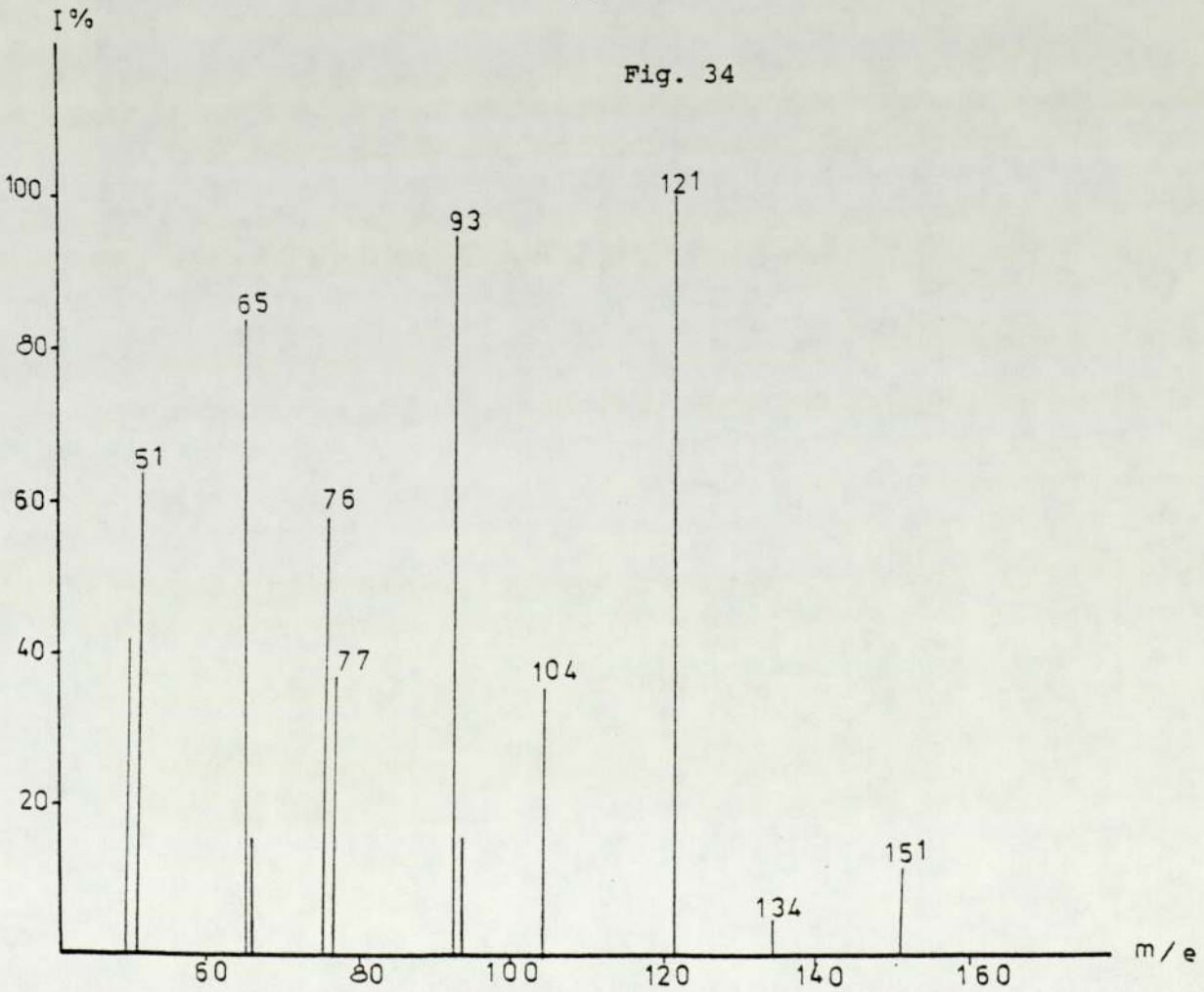


Fig. 34



The uv and ir spectra of o-nitrosobenzoic acid isolated from the photolysis experiments were identical to those of a prepared sample.

No quinoline or 1,2,3,4-tetrahydroquinoline were detected (tlc) in the photolysate from (3.23) and the fate of this residue remains, as yet, a mystery.

3.5 Mechanism of Reaction

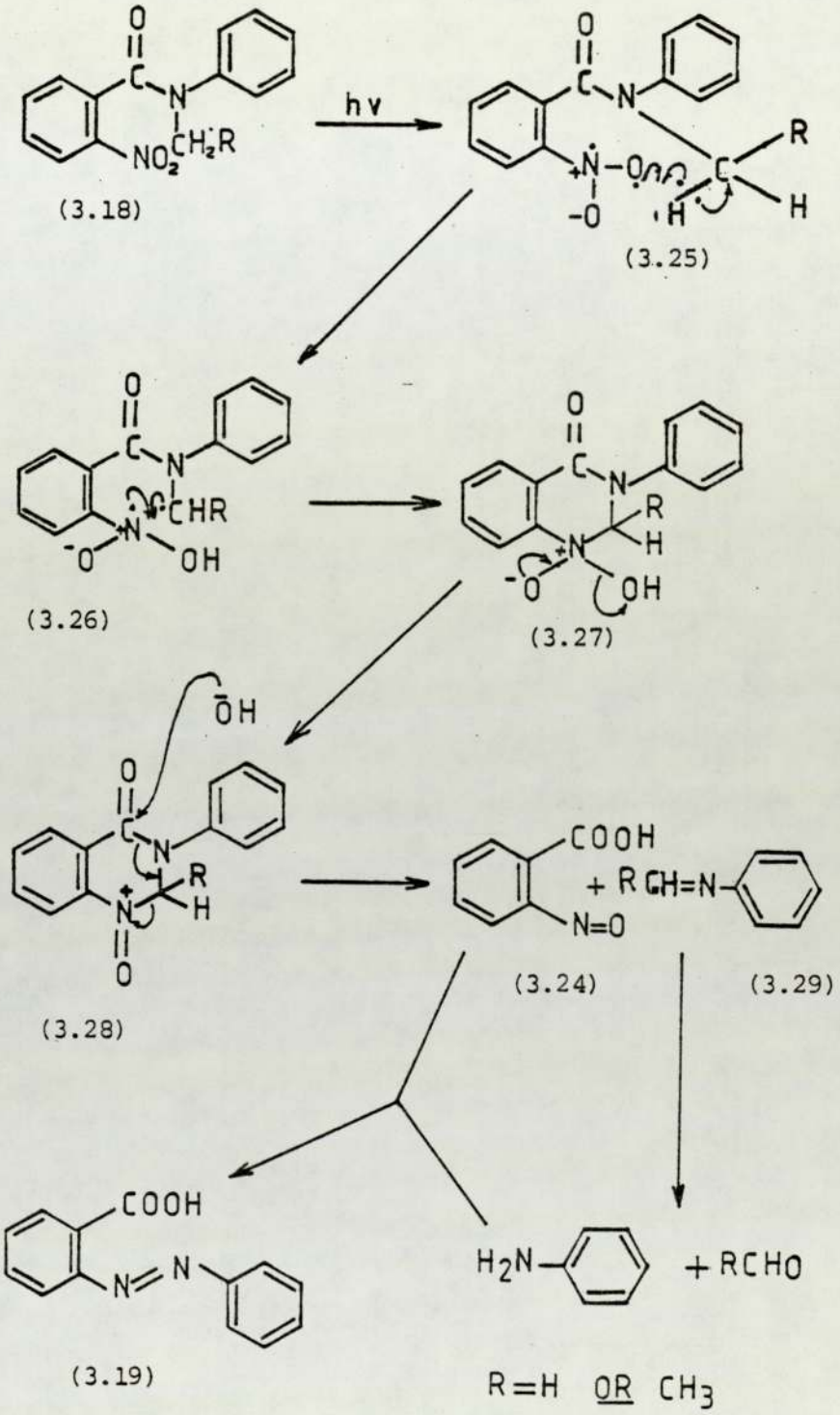
The photo-rearrangement of o-nitrobenzanilides having a free amidic NH group was envisaged by Gunn and Stevens[125] as proceeding *via* an initial hydrogen abstraction of the amidic NH by the excited nitro group. With the secondary amide site blocked by an N-alkyl group clearly a different mechanism must apply in the present cases.

A plausible mechanism to account for the formation of the major photolytic product azobenzene-2-carboxylic acid (3.19) and the minor product o-nitrosobenzoic acid (3.24) is summarised in (scheme 18).

This mechanism involves hydrogen abstraction of an α-hydrogen of the alkyl group followed by radical recombination to afford the 1-hydroxyquinazolone-1-oxide (3.27). This could conceivably undergo ring opening *via* an intimate ion-pair (3.28) to afford o-nitrosobenzoic acid and the appropriate anil (3.29). Hydrolysis of the anil under the conditions of the photolysis could afford aniline (which was detected in the photolysis of (3.18, R = H or Me). Condensation of the o-nitrosobenzoic acid and the liberated aniline would then afford the observed azobenzene-2-carboxylic acid (3.19). (It was independently shown that aniline and o-nitrosobenzoic acid react together to form o-nitrosobenzoic acid under the conditions of the photolysis.)

However, to set against this evidence neither of the anils (3.29; R = H or Me), or formaldehyde (from 3.18b; R = H) or acetaldehyde (from 3.18a; R = Me) were detected in the photolysis products. The mechanism advanced in (scheme 18) envisages the formation of azobenzene-2-carboxylic acid as involving an

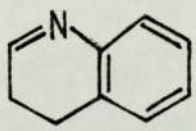
(scheme 18)


hν
(3.18)
(3.25)
(3.26)
(3.27)
(3.28)
(3.24)
(3.29)
(3.19)
COOH
N=O
+ RCH=N
H_2N
+ RCHO
R=H OR CH_3

intermolecular reaction between o-nitrosobenzoic acid and aniline. By incorporating a different arylamine in the photolysis mixture this point could be checked, although it was not performed in the present work.

In contrast irradiation of the tetrahydroquinoline (3.23) in ethanol led to the formation of o-nitrosobenzoic acid (3.24) as a major photo-product.

If the o-nitrosobenzoic acid is formed by the mechanism outlined in (scheme 18), then 3,4-dihydroquinoline (3.30) should



(3.30)

be formed as a by-product. This cannot, of course, hydrolyse to an arylamine as in the foregoing examples, and hence no azobenzene-2-carboxylic acid was formed. However, neither the dihydroquinoline (3.30) or its oxidised aromatic form, quinoline, were detected in the photolysis products.

The plethora of other coloured photo-products from (3.18; R = H or Me) are as yet unexplained, in particular the formation of 2'-hydroxyazobenzene-2-carboxylic acid (3.20). This product has previously been shown to be the major photo-product from o-nitrobenzanilide (3.6).[125] The starting N-alkyl-o-nitrobenzanilides (3.18; R = H or Me) were rigorously checked for the presence of o-nitrobenzanilide, but this was not shown to be a contaminant. However, o-nitrobenzanilide (3.6) could arise in

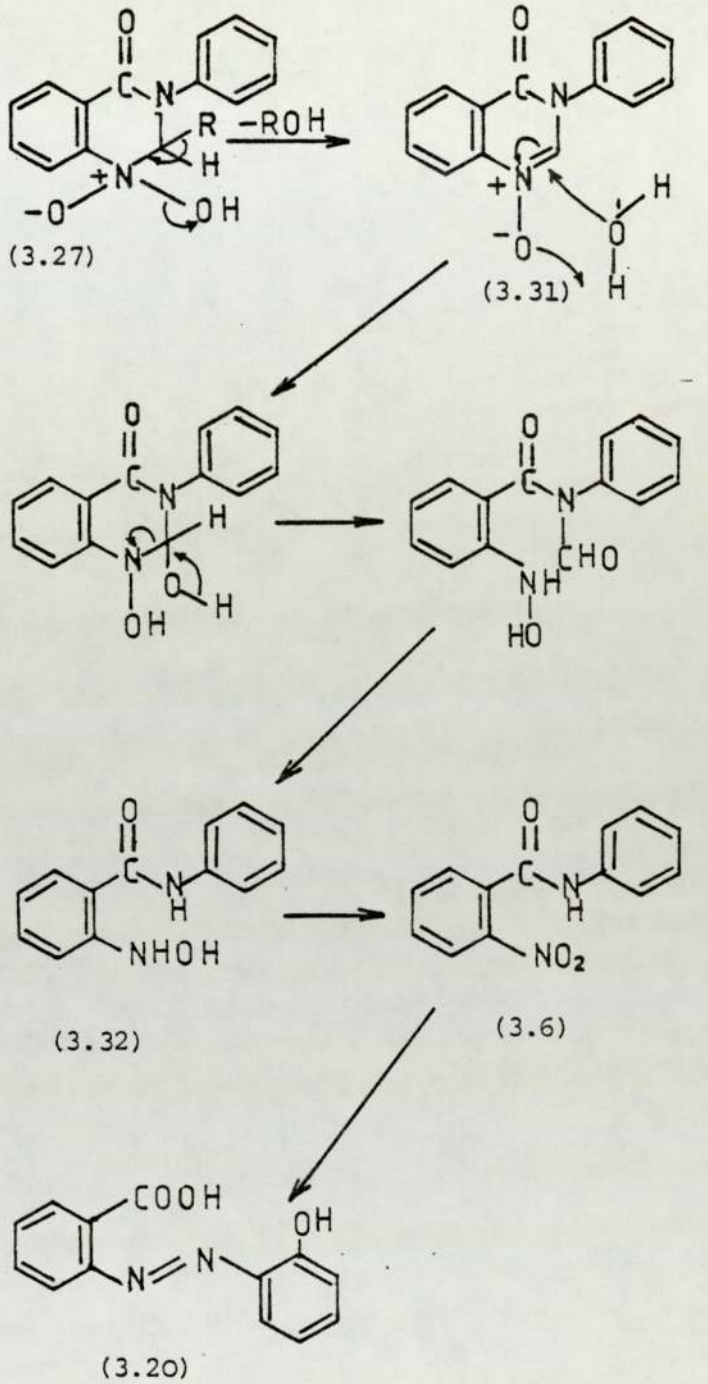
the photolysis by two pathways:

(i) Photochemical dealkylation of the N-alkyl-o-nitrobenzanilide (3.18)

(ii) Photo-oxidation of o-hydroxylaminobenzanilide (3.32). The latter hydroxylamine may be formed from the proposed intermediate 1-hydroxyquinazolone-1-oxide (3.27) according to (scheme 19).

In this process the 1-hydroxyquinazolone (3.27) undergoes elimination of either water or methanol to afford an intermediate quinazolone-oxide (3.31) which then suffers successively hydration, ring-opening and hydrolysis. If the hydroxylamine (3.32) is an intermediate it is possible that it may be oxidised to o-nitrobenzanilide (3.6). It may also partake in bimolecular reactions to give the range of (as yet) unidentified coloured products also formed in these photolyses.

(scheme 19)



R = H OR CH_3

CHAPTER IV

SEARCH FOR 2-NITRO O TOLUIDIDE IN THE URINE OF PATIENTS TREATED WITH METHAQUALONE

Search for 2-Nitrobenz-o-toluidide in the Urine of Patients Treated with Methaqualone

4.1 Introduction

Methaqualone (4.1; 2-methyl-3-o-tolylquinazol-4(3H)-one) is a non-barbiturate sedative hypnotic used clinically for treatment of insomnia and anxiety.[137] Recently, methaqualone abuse has increased among youthful drug users and evidence has been published suggesting that methaqualone can induce physical dependency similar to that induced by barbiturates.[138]

The metabolic fate of methaqualone has been extensively studied in man and other species.[139-142] The major metabolic route appears to be C-oxidation, mainly by the hepatic microsomal enzyme system, to a number of monohydroxy derivatives (Table 18) which are subsequently conjugated with β-glucuronic acid.

Akagi et al[139] showed that the main urinary metabolite in rabbit was the glucuronide of the 2'-hydroxymethyl derivative (compound 2, Table 18). Nowak et al[143] identified this metabolite and in addition the 3'-, 4'-, 6'- and 8-hydroxy derivatives (compounds 3, 4, 10 and 8 respectively, Table 18) as their glucuronides in urine of the rat, rabbit, dog and rhesus monkey. Preuss et al[144] showed that in the urine of man the 2'-hydroxymethyl-, 3'-, 4'-, and 6-hydroxy derivatives were present, but concluded that the 2-hydroxymethyl derivative (compound 1) was absent. Preuss and Hassler[145] and Preuss et al[146] subsequently identified two additional minor metabolites in man using ir, mass spectroscopy and nmr data. These were a dihydroxy derivative, 6-hydroxy-2-methyl-3-(3'-hydroxy-2'-methylphenyl)quinazol-4(3H)-one and a

methoxy compound (Table 18, compound 12).

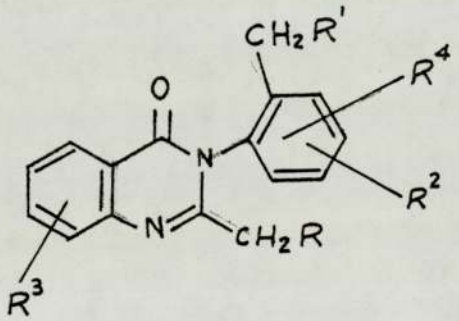
Using gc-ms to examine the trimethylsilylated urinary metabolites of three people intoxicated with Mandrax, Bonnichsen et al[147] were able to identify five metabolites. Also Burnett et al,[148] investigating the fate of methaqualone in healthy volunteers, reported the presence of three major metabolites (compounds 2, 3, 4; Table 18) and three minor metabolites (compounds 1, 6, 8; Table 18). They also deduced the presence of an 8-hydroxyquinazolone for the first time.

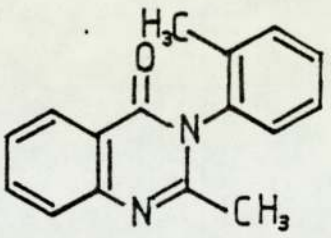
In addition to the C-oxidation metabolic route it is possible for methaqualone to undergo N-oxidation. Murata et al[149] claimed that in man receiving oral doses of the drug, one of the urinary metabolites was 2-nitrobenz-o-toluidide (4.3). It was observed that incubation of methaqualone with the 9000 x g supernatant fraction of rabbit liver gave methaqualone-N-oxide as one of the two major metabolites. Incubation of the N-oxide with the 9000 x g fraction under nitrogen or without air resulted in 50-60% reduction of the N-oxide to methaqualone and no 2-nitrobenz-o-toluidide was produced. In contrast,[150] when the incubation was carried out under oxygen 2-nitrobenz-o-toluidide was formed. Oral administration of the N-oxide (4.2) to rabbits gave rise to the urinary excretion of (4.3), the 2'-hydroxymethyl derivative (compound 2; Table 18) and methaqualone (4.1). Murata et al[151] concluded that in vivo methaqualone-N-oxide (4.2) may be an intermediate in the metabolism of methaqualone, but was not itself excreted in urine. On the contrary, methaqualone-N-oxide has been detected in human urine by Renolds et al.[152] It has been identified by gc-ms. To add yet more confusion, Renolds et al[152] failed to detect 2-nitrobenz-o-toluidide as a human urinary metabolite of methaqualone.

In view of the frequent occurrence of N-oxides as metabolites of many tertiary amine and heterocyclic drugs, and the possibility of the presence of the corresponding ring-opened nitro compound as a metabolite of methaqualone it was decided that a re-investigation of the N-oxidation of the drug in man would be valuable.

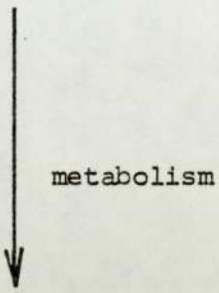
Table 18

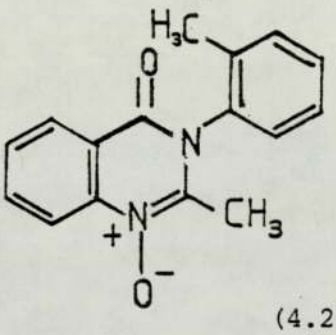
Formulas of methaqualone and its C-Oxidation Urinary Metabolites

No.	Methaqualone and Metabolites	R	R^1	R^2	R^3	R^4
	Methaqualone	H	H	H	H	H
1		OH	H	H	H	H
2		H	OH	H	H	H
3		H	H	3'-OH	H	H
4		H	H	4'-OH	H	H
5		H	H	H	5-OH	H
6		H	H	H	6-OH	H
7		H	H	H	7-OH	H
8		H	H	H	8-OH	H
9		H	H	5'-OH	H	H
10		H	H	6'-OH	H	H
11		H	H	3'-OH	6-OH	H
12		H	H	5'-OCH_3	H	4'-OH

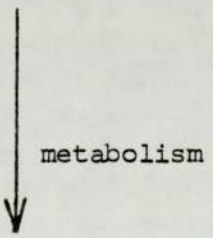


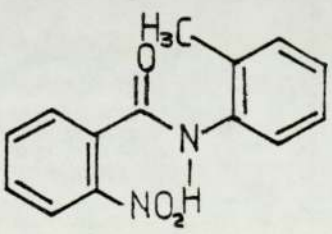
(4.1) Methaqualone





(4.2)





(4.3)

4.2 N-Oxidation of Methaqualone

Murata and Yamamoto[150] reported that oxidation of methaqualone with hydrogen peroxide in acetic acid afforded the N-oxide (4.2). However, when this procedure was repeated in the present work, even under mild conditions, the end product was always 2-nitrobenz-o-toluidide (4.3) which was identical in physical and spectroscopic characteristics to an authentic sample prepared from o-nitrobenzoyl chloride and o-toluidine. It is probable that the N-oxide is indeed the initial product and that it then suffers hydrolytic (as shown in scheme 20) or possibly acetolytic ring-opening. The hydroxylamine derivative (4.4) formed by ring-fission could then undergo oxidation to the nitroarene and deacetylation under the conditions of the reaction to afford 2-nitrobenz-o-toluidide (4.3).

When oxidation of methaqualone was attempted under milder conditions using m-chloroperbenzoic acid in dichloromethane in the dark for 7 days, a new product in addition to the nitrotoluidide was detected in trace amount on tlc. This fluorescent compound had an Rf value (0.24) on alumina which agreed well with that reported for methaqualone-N-oxide.[150] In addition the uv and fluorescence spectra (Table 19) were entirely consistent with data recorded by Murata and Yamamoto for the N-oxide.[150] However, the N-oxide was produced as a minor oxidation product only and insufficient material

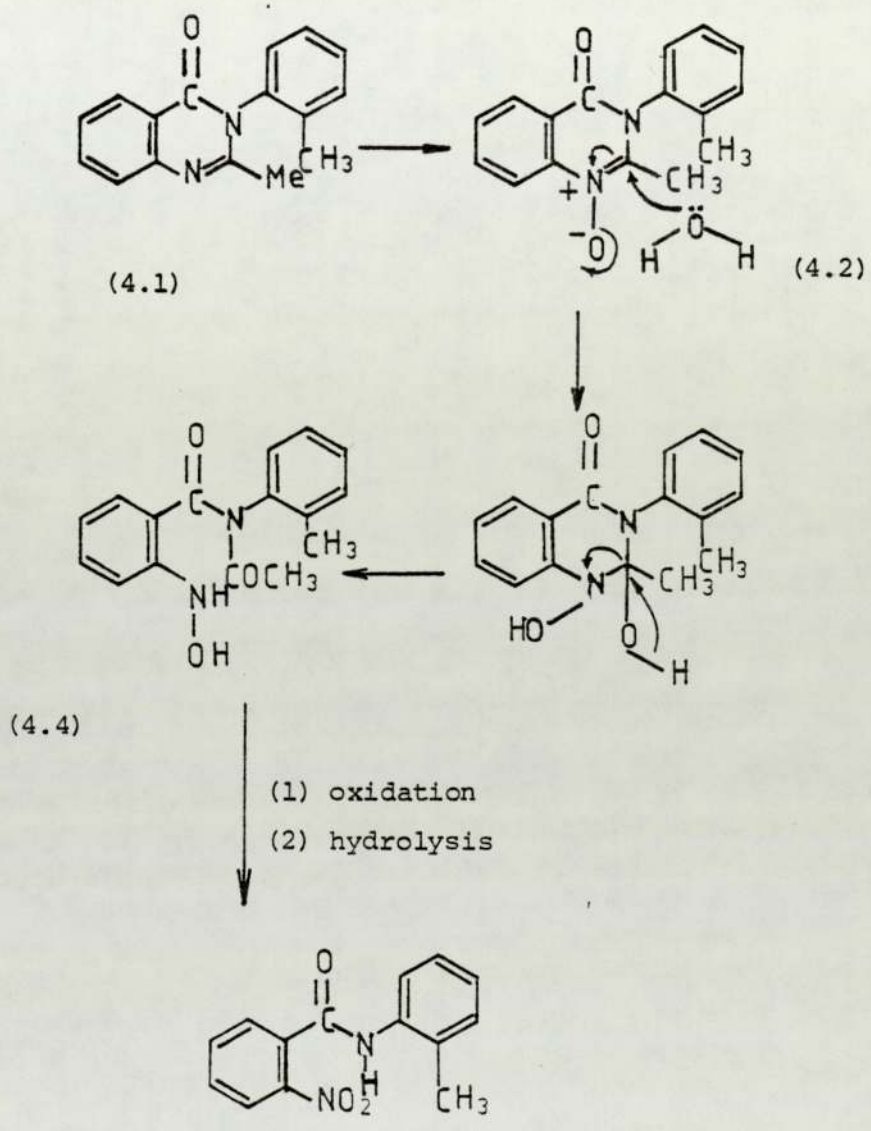
Table 19

Uv Absorption and Fluorescence Emission of Methaqualone N-oxide

Spectral Character	Reported data	Found data
Uv	315 nm	315 nm
Fluorescence emission	325 nm 487 nm	325 nm 487 nm

was recovered to examine the properties of this intriguing compound.

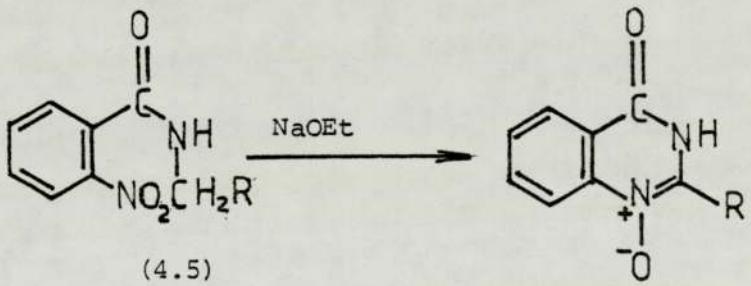
(scheme 20)



(4.1) (4.2) (4.4) (4.3)

The stability of this N-oxide to oxidising condition, and its behaviour in the presence of nucleophiles would make an interesting topic for study.

An alternative synthesis of methaqualone N-oxide suggested itself in work published by Tennant and Vaughan.[153] These authors showed that substituted nitrobenzamides (4.5; R = CN or COPh) are cyclised in sodium ethoxide to quinazolone-N-oxides (4.6; R = OEt). It was suggested that the 2-ethoxy substituent may be introduced by nucleophilic displacement of the initial substituent in the 2-position in the N-oxides (4.6). This site would be activated to nucleophilic attack by the adjacent N-oxide grouping.

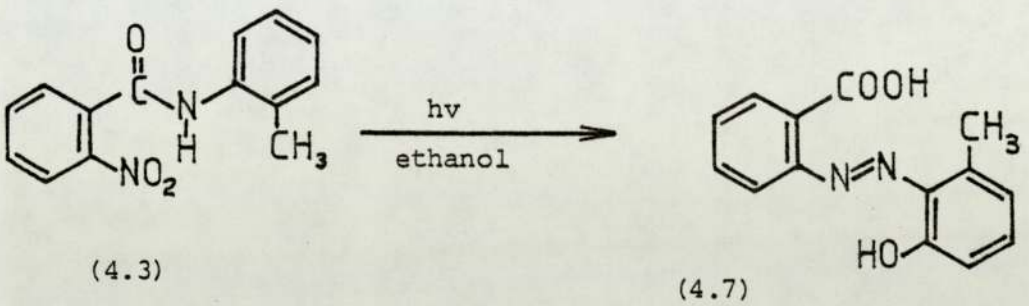


(4.5) (4.6)

However when N-methyl- or N-ethyl-2-nitro-benzanilides (3.18) were refluxed in sodium ethoxide absolutely no reaction occurred. Similarly boiling pyridine or piperidine were without effect on these anilides. Evidently an alectron-attracting substituent R in (4.5) is required to activate the adjacent methylene group for cyclisation to occur.

4.3 Detection of 2-Nitrobenz-o-toluidide in Urine

As has been reported[125] the nitrotoluidide (4.3) is extremely photosensitive even in the dry state. 2-Nitrobenz-o-toluidide (4.3) when irradiated rearranges to give the azocarboxylic acid (4.7) (see Chapter III).[125]



(4.3) (4.7)

From a solution of (4.3) in dichloromethane (15 mgm/l), a 5 µl spot can be visualised on a silica gel_{254} tlc plate by the development of the red colour of (4.7) when the plate is exposed to ultraviolet/visible light. On the other hand, a more conventional visualisation of the nitro compound by reduction on the plate with stannous chloride-hydrochloric acid, followed by spraying with an amine reagent was less sensitive.

Gas liquid chromatography (glc) is widely used for the separation and identification of organic compounds which are volatile and have sufficient thermal stability to survive the process of vaporisation and passage through a chromatography column. The highly polar nitro group does not normally fulfill these requirements. It is also noted that the choice of a glc detector depends on the sensitivity required.

The flame ionisation detector (FID) is sensitive at the nanogram level and the electron capture detector (ECD) is sensitive to the picogram level. In its favour FID is robust and simple to

operate whereas ECD requires more skill and gives poor results when mishandled. It was anticipated that the expected physiological levels of 2-nitrobenz-o-toluidide (4.3) in urine - if indeed it were present - would require the greater sensitivity of ECD.

Detection of (4.3) and (4.1) in a mixture using glc

a) Flame ionisation detector

Sample preparation

Methaqualone used for this work was kindly provided by The Boots Company Ltd., Nottingham, and 2-nitrobenz-o-toluidide was prepared according to the reported method (Experimental Section) and tested for purity by tlc, m.p. and spectral characteristics.

A synthetic mixture of methaqualone (4.1) and 2-nitrobenz-o-toluidide (4.3) was accurately weighed in acetone. A series of dilutions were prepared in the same solvent varying from 0.1 mg/ml to 1 mg/ml of both components.

Glc conditions

A semi-polar column was initially investigated and a 1 m x 4 mm I.D. 3% Se 30 column was prepared and conditioned at an oven temperature of 265°. Solutions of (4.1) and (4.3) were subjected to glc analysis using FID. The injected solution of (4.1) and (4.3) (2 µl) were separated with reasonable retention times (Table 20).

Table 20

Observed retention times of (4.1) and (4.3) on glc analysis

Compound	Column material	Retention time (mins)
Methaqualone (4.1)	3% Se30	2.2
2-Nitrobenz-o-toluidide (4.3)	3% Se30	3.4
Methaqualone (4.1)	5% OV25	2.8
2-Nitrobenz-o-toluidide (4.3)	5% OV25	4

The use of a semi-polar column was unsatisfactory with the nitro compound (4.3) which failed badly. A 1 m x 4 mm I.D. 5% OV 25 column was prepared and conditioned. The use of this more polar column under the experimental conditions improved the resolution of the nitro compound (4.3).

A plot of concentration versus peak area gave a linear relationship for both methaqualone and 2-nitrobenz-o-toluidide (Fig. 36).

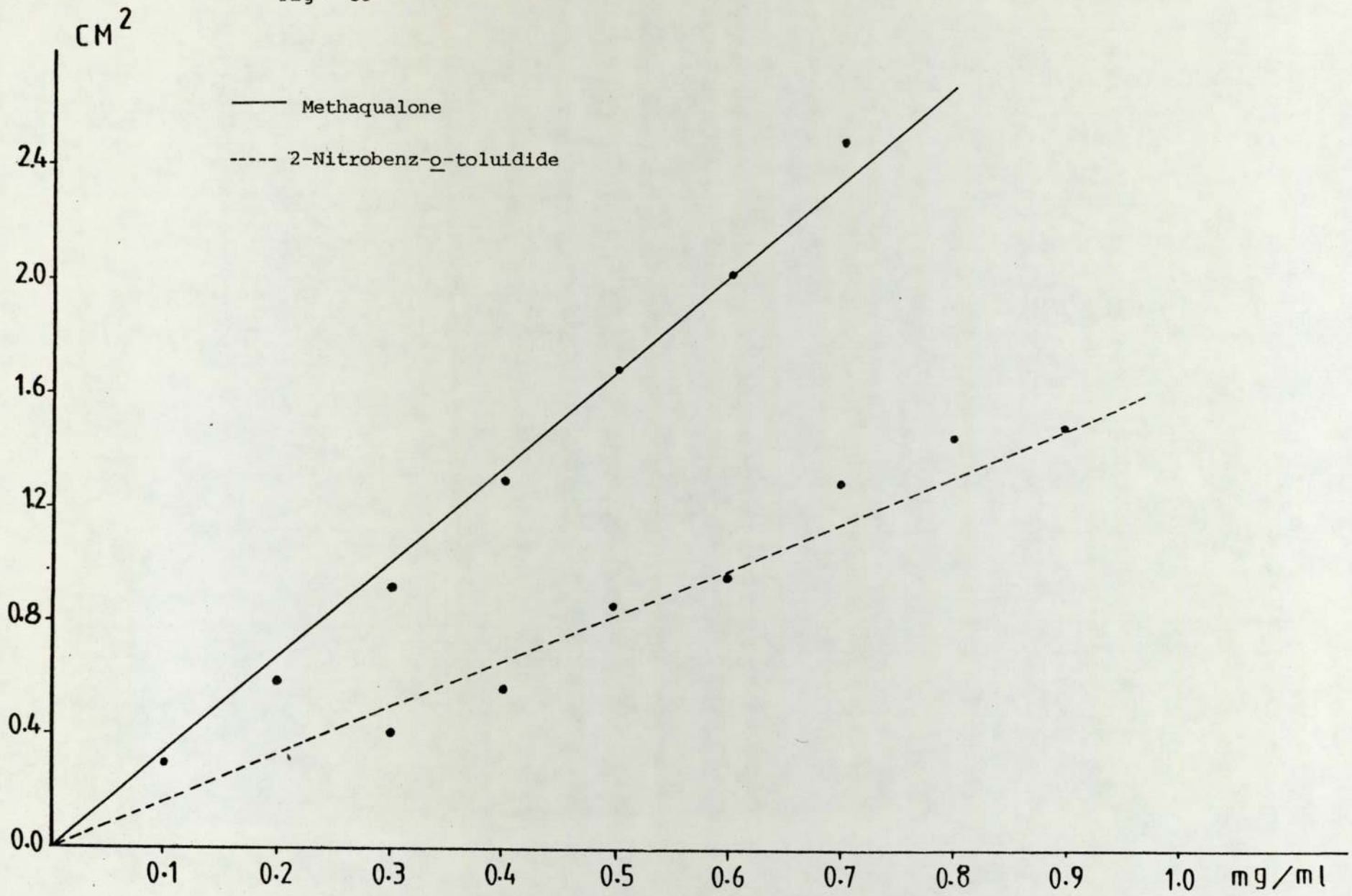
A synthetic mixture of methaqualone (4.1) and 2-nitrobenz-o-toluidide (4.3) in urine was then prepared. The mixture was extracted using dichloromethane and the problem of emulsion formation overcome by saturating the aqueous fraction with ammonium sulfate. The final solution in dichloromethane was adjusted to known volume and dilutions suitable for glc analysis were prepared (0.5 mg/ml of each (4.1) and (4.3)). Glc conditions were the same as previously mentioned.

Sample extracted from blank urine and the synthetic urine solutions of (4.1) and (4.3) were subjected to glc analysis using FID. Percent recoveries of each component were 97% of the theoretical amount.

b) Electron Capture Detector

Sample preparation was accomplished in the same manner as previously but the final extraction solution in dichloromethane was evaporated to dryness. The residue was dissolved in acetonitrile of analytical grade. A series of dilutions was also prepared in acetonitrile (note: ECD is sensitive towards halogenated compounds). Then the solutions in acetonitrile were subjected to glc investigation. Glc conditions were the same as under FID but only the polar column (5% OV25) was used.

Fig. 36


CM 2
Methaqualone
2-Nitrobenz-o-toluidide
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
mg/ml

By the use of ECD the level of detection of 2-nitrobenz-o-toluidide went down to 0.05 ng/ml instead of 3 mg/ml and the methaqualone to .01 ng/ml.

Detection of 2-nitrobenz-o-toluidide in urine of a patient receiving methaqualone

Methaqualone tablets (250 mg methaqualone) and the urine of an adult male patient who had received a combination of Mandrax, Largactil and Welldorm (one dose at night for 2 weeks before test, Table 21) were kindly supplied from "All Saints Hospital", in Birmingham.

Table 21

Details of drugs taken by patient

Tablet commercial name	drug ingredient	amount in mg
Mandrax	Methaqualone	250
	Diphenhydramine hydrochloride	25
Largactil	Chloropromazine hydrochloride	100
Welldorm	Dichlorophenazine	650

Urine samples were collected early in the morning. An aliquot of 500 ml was taken and extracted with dichloromethane. The dichloromethane extract was collected and evaporated to dryness. The residue was dissolved in acetonitrile (100 ml). The sample was studied on tlc using silica gel_{254} (0.25 mm; chloroform-methanonol; 9-1). The chromatogram showed about 15 overlapping spots. The use of methaqualone and 2-nitrobenz-o-toluidide references revealed the presence of methaqualone among the overlapping spots (Rf =) but there was no 2-nitrobenz-o-toluidide present. The numerous other unidentified spots are presumably metabolites of either

methaqualone or the other component of the combination together with their metabolites. The sample was also investigated on glc using FID and ECD but the resolution of the peaks was very poor presumably again, because of the numerous metabolites formed.

Dosage of volunteers

Volunteers who had not previously received any drug for at least two weeks each received an oral dose of 500 mg methaqualone (Melsed tablets) at 10.00 h. Urine samples were collected 12 hours after dosage and stored in the dark. A sample of urine was also collected from each volunteer immediately before the drug was administered.

Aliquots of 500 ml of both blank and test urines were extracted using dichloromethane; the final extraction solutions were evaporated and the residues dissolved in acetonitrile. The blank urine sample and the dosed urine were studied by tlc (0.25 mm; silica gel_{254}; chloroform-methanol; 9-1) against references of methaqualone and 2-nitrobenz-o-toluidide. The dosed urine sample revealed the presence of methaqualone (Rf = 0.4) but not 2-nitrobenz-o-toluidide.

The test sample of urine was then subjected to glc analysis using ECD under the glc conditions mentioned before (5% OV25 column). The chromatogram revealed the presence of unchanged methaqualone and some of its metabolites: however no 2-nitrobenz-o-toluidide was detected.

4.3 Conclusion

The results presented in this work are clearly at variance with those previously reported. 2-Nitrobenz-o-toluidide (4.3), supposedly a major metabolite of the drug methaqualone (4.1), was not detected in the urine samples of volunteers either by tlc or

glc. Because of the sensitivity of the ECD it is reasonable to conclude that 2-nitrobenz-o-toluidide is not a metabolite of methaqualone in humans.

PART III

CHAPTER V

EXPERIMENTAL

Notes:

Unless otherwise stated:

(1) All melting points are uncorrected.

(2) "Ethanol" refers to 95% ethanol.

(3) Ultraviolet spectra were recorded in ethanol on a Unicam SP8000 spectrophotometer.

(4) Infrared spectra were recorded in the form of KBr discs, or solutions in chloroform, carbontetrachloride or dimethylsulphoxide, on a Unicam SP200.

(5) Mass spectra were recorded on a Micromass 12 mass spectrometer operating at 70 eV with an inlet temperature in the range of 200-300°.

(6) Glc analyses were carried out on a Pye Unicam GCV chromatograph.

(7) Photolyses were conducted with an unfiltered 100 W medium-pressure arc in 1 litre Hanovia photochemical reactor.

5.1 Infrared Spectroscopy of Substituted Nitrobenzenes

Samples used were either commercial samples of analytical grade, or research samples prepared in our laboratory; melting points were checked before use as an indication of purity.

The spectra were first scanned rapidly on a Unicam SP200, then bands of primary interest (between 1667 cm^{-1} to 1250 cm^{-1}) were expanded on a Grubb Parsons spectromaster with a scan speed of 1 μ/16 minutes.

Absorption frequencies were converted to wave numbers (cm^{-1}) according to conversion tables.[84] Wave numbers were accurate to ± 1 cm^{-1}, except for very wide bands.

5.2 Synthesis and Photochemical Properties of Nitrobenzanilides

o-Nitrobenzoyl chloride

A solution of o-nitrobenzoic acid (0.5 M) in benzene (280 ml) was distilled to remove the benzene-water azeotrope (50 ml). The mixture was cooled and thionyl chloride (40 ml; 0.55 M) was added. The mixture was refluxed for 2 hours then excess thionyl chloride was distilled (ca 50 ml). Each 1 ml of residual benzene contains 0.5 g of o-nitrobenzoyl chloride.

o-Nitrobenzamide

A solution of o-nitrobenzoyl chloride (1 mol. equiv.) was stirred with excess of cold concentrated aqueous ammonia and ice for 0.5 hours. The precipitate was filtered off, washed with water and dried to give 2-nitrobenzamide (95%), m.p. 174-175° (cream needles, from ethanol).

Similarly prepared from o-nitrobenzoyl chloride, the appropriate amine (1 mol. equiv.) and excess of aqueous 2N-sodium hydroxide at 20°C were the following N-substituted o-nitrobenzamides:

N-methyl-N-phenyl-; m.p. 107-108°

N-ethyl-N-phenyl-; m.p. 92-93°

N-o-tolyl-; m.p. 175-176°

N-(o-Nitrobenzoyl)-1,2,3,4-tetrahydroquinoline

This compound was prepared from o-nitrobenzoyl chloride and 1,2,3,4-tetrahydroquinoline as above. The resulting product had m.p. 142-145° (Lit.,[132] m.p. 152°).

Photolysis of N-ethyl-o-nitrobenzanilide (3.18a)

N-Ethyl-o-nitrobenzanilide (5 g) was dissolved in ethanol (1 Litre) and photolysed for 48 hours. The solution rapidly darkened to a deep brown colour (2 hours). The photolysate was

concentrated to 100 ml. Chromatographic separation of this solution on a neutral alumina column led to recovery of starting material (75%) which was eluted with ethanol, leaving a bright orange immobile band at the top of the column. The orange photoproduct was desorbed from the extruded column with aqueous 2N-sodium hydroxide (30 ml) and acidic material reprecipitated with 10 N-hydrochloric acid. The precipitate was extracted (continuous extract) with acetone and refractionated on a silica gel column (using chloroform then chloroform/methanol as eluting solvents). The first fraction on evaporation gave bright orange needles of azobenzene-2-carboxylic acid from aqueous ethanol (m.p. 88°); the second compound eluted, 2'-hydroxyazobenzene-2-carboxylic acid crystallised as maroon plates from benzene (m.p. 202°). Chemical structures were confirmed by spectral properties (see pp. 86).

Subsequent examination on tlc plates of the photolysate in both cases on silica gel (0.25 mm; ethylacetate-methanol-aqueous ammonia: 15-4-1) showed the presence of at least 10 other components including o-nitrosobenzoic acid, unreacted starting material and highly coloured components remaining near the origin.

Hydrogenation of azobenzene-2-carboxylic acid

The azo compound (3.19) dissolved in ethanol was hydrogenated over a 10% palladium on charcoal catalyst. Two mol. equiv. of hydrogen was absorbed, and the two products were identified as anthranilic acid and aniline (tlc examination comparing with references).

Isolation and identification of aniline from the photolysate of (3.18a)

After isolation of the red products, the photolysate was

refractionated on a silica gel column using chloroform as eluting solvent. The first fraction from the column (25 ml), which contained aniline together with N-ethylaniline, was shaken with 5N- hydrochloric acid. Aniline was separated as its hydrochloride salt. The base was liberated with ammonia, extracted into chloroform, and reapplied to a tlc plate (silica gel, 0.25 mm; chloroform/methanol). The tlc plate was sprayed with cold 6N-hydrochloric acid then sodium nitrite solution; and finally the plate was sprayed with a cold solution of 2-naphthol in an excess of 2N- NaOH solution: a brilliant red dye was produced from the isolated aniline which had an Rf value identical with a reference sample of aniline.

Photolysis of N-methyl-o-nitrobenzanilide (3.18b)

N-Methyl-o-nitrobenzanilide (3.18b) (5 g) was photolysed in ethanol (1 litre) for 48 hours and worked up as described above. Investigation of the photolysate was also carried out in the same manner to afford the same major photolytic products (3.19) and (3.20) (see pp. 92) and other minor compounds (Table 19, pp.91).

Preparation of 2-methyl-3-phenylquinazol-4(3H)-one

This compound was prepared by the acetylation of o-amino-benzanilide with acetic anhydride according to the literature method. It crystallised as yellow prisms from ethanol, m.p. 143-145 (Lit m.p. 145-147°).[154]

Photolysis of N-(o-nitrobenzoyl)-1,2,3,4-tetrahydroquinoline

This compound (3 g) was photolysed in the usual manner in ethanol (1 litre) for 48 hours. The solution changed to bright yellow (after 4 hours). Chromatographic fractionation on a neutral alumina column led to the recovery of starting material

(80%) which was eluted with ethanol, leaving a green immobile band on the column. The green photoproduct was desorbed from the extruded column with aqueous 2N-sodium hydroxide (30 ml) and reprecipitated with 10N-hydrochloric acid. The precipitate was dissolved in ethanol, and fine green crystals were deposited on the walls of the flask (m.p. 207-10° decomp, from ethanol). The photoproduct was identified as o-nitrosobenzoic acid, from its spectral characters, and its identity with an authentic reference sample (see below).

Preparation of o-nitrosobenzoic acid

This acid was prepared either by oxidation of anthranilic acid by permonosulfuric acid,[155] or by photolysis of o-nitrobenzaldehyde in benzene.[135] The purity of o-nitrobenzoic acid prepared by the latter method was superior to the former method.

5.3 Search for 2-Nitrobenz-o-toluidide in the Urine of Patients Treated with Methaqualone

Qualitative identification of 2-nitrobenz-o-toluidide

A solution of (4.3) was prepared in ethanol in concentrations varying from 5 to 50 mg/litre. A spot of 5 µl of each concentration was applied to a tlc plate (silica gel_{254}; 0.25 mm, chloroform-methanol; 9-1). The spots were visualised by exposing them at 254 nm for 3 hours. The lowest concentration detectable by this method was the solution of 15 mg/l.

Preparation of Methaqualone-N-oxide

Two methods were applied for the preparation of methaqualone-N-oxide:

a) Oxidation of methaqualone with hydrogen peroxide in glacial acetic acid according to the reported method.[151]

b) A solution of methaqualone (61 g, 0.255 mol) dissolved in dichloromethane (1.5 L) was added portion-wise to m-chloroperbenzoic acid (82.5 g, 0.271 mol) over 2 hours. The mixtures were stirred at room temperature in the dark for 7 days. The solution was washed with 2N-sodium hydroxide to remove any excess of free carboxylic acid and then dried by shaking with anhydrous sodium sulphate and the solvent evaporated under vacuum.

Methaqualone-N-oxide was separated by preparative tlc (0.75 mm; alumina; chloroform-methanol; 9-1). The N-oxide band was visualised at 254 nm (Rf = 0.24). An ethanolic extract of this band was used for uv and fluorescence emission identification.

Quantitative identification of methaqualone (4.1) and 2-nitrobenz-o-toluidide (4.3)

A synthetic solution of (4.1) and (4.3) was prepared in acetone in concentrations varying from 0.1 mg/ml to 1 mg/ml for

each ingredient. The solutions were separated by glc using a 3% Se 30 or a 5% OV 25 column on supasorb (AW-HMDS) at an oven temperature of 265°. The other glc parameters were as follows:

Injection temperature	350°
Nitrogen flow rate	60 ml/min FID
Air pressure	5 lb/in^2 "
Hydrogen pressure	16 lb/in^2 "

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