Aspects of the biological activity of the schistosomicide oxamniquine

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ASPECTS OF THE BIOLOGICAL ACTIVITY OF THE SCHISTOSOMICIDE OXAMNIQUINE

by

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A Doctoral Thesis

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For Gaju and Paul

"Now faith is being sure of what we hope for and certain of what we do not see."
Heb. 11:1
(The Holy Bible, New International Version, © International Bible Society)

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"...thanks to be God! He gives us the victory through our Lord Jesus Christ."
1Cor. 15:57 (The Holy Bible, New International Version)

Summary

Oxamniquine, 6-hydroxymethyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, is a potent schistosomicide used clinically in the treatment of infections due to *Schistosoma mansoni.* Schistosomiasis is the second most important tropical disease after malaria. Although oxamniquine is relatively well tolerated, severe central nervous system (CNS) effects characterized by convulsions, have been reported in a small percentage of the population treated with this drug.

The specific aims of the project were firstly to synthesize structural variants of oxamniquine by chemical modification of the molecule and secondly to investigate the effect of structural modification on the biological activity, in particular any CNS activity.

Several novel analogues of oxamniquine were obtained by structural modification of the molecule. The 6-CH_2OH was replaced by :- CO_2H, $CH(OH)CH_3$, $C(O)CH_3$, $CH=CH_2$, $CH(OH)CH_2OH$, $CH=CHOCH_3$ and $CH=CHSCH_3$. Several unexpected reactions were observed, for which possible mechanisms have been proposed to explain this unusual chemistry and reactivity inherent in the oxamniquine molecule. These include the oxidative cleavage of the methoxyvinyl group with perchloric acid and an intramolecular ylid rearrangement for the reaction of oxamniquine aldehyde with dimethylsulfonium methylide to yield the methylthiovinyl compound and the dihydroxyethanyl derivative.

The CNS depressant activity of oxamniquine, in doses exceeding therapeutic drug levels, has been demonstrated in the anaesthetized mouse model. The screen, developed in attempt to demonstrate the CNS excitatory effects of oxamniquine, involved the evaluation of the effects of the drug on catechol-induced convulsant activity in the anaesthetized mouse. However, although the convulsant activity of oxamniquine has not been demonstrated, it has been confirmed that the depressant effect of oxamniquine is central in origin. The CNS effects of the 6-carboxylic acid, the major metabolite of oxamniquine, have also been investigated to determine its contribution to the central effects of oxamniquine. The acid was shown to be devoid of any central activity in the model. The CNS depressant activity of the secondary alcohol homologue of oxamniquine, 6-(1-methyl hydroxymethyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, has been demonstrated in the model.

The possible antischistosomal activity of the analogues has been evaluated using an *in vitro* model system. Although screening for antischistosomal activity invariably involves investigating the effect of test compounds on the parasite, recent reports have shown a good correlation between inhibition of macromolecular biosynthesis and antischistosomal activity. The effects of the compounds on the incorporation of radiolabelled uridine into macromolecules has been investigated using isolated hamster liver nuclei. Preliminary results have demonstrated the ability of oxamniquine to inhibit uridine incorporation into macromolecules. However, the effect of the analogues on macromolecular biosynthesis and therefore their potential antischistosomal activity could not be confirmed.

The chemistry, CNS effects and the effects on macromolecular biosynthesis of oxamniquine and analogues are discussed.

Abbreviations

Ac acetyl
ADME absorption, distribution, metabolism and excretion
ATP adenosine 5'-triphosphate
BBB blood brain barrier
CNS central nervous system
CI chemical ionization
CTP cytidine 5'-triphosphate
DMF dimethyl formamide
DMSO dimethyl sulfoxide
DNA deoxyribonucleic acid
DPM disintegrations per minute
EEG electroencephalogram
EI electron impact
GABA gamma-amino butyric acid
GTC generalized tonic-clonic seizures
GTP guanosine 5'-triphosphate
i.m. intramuscular
i.p. intraperitoneal
IR infrared
i.v. intraveneous
LDA lithium di-isopropylamide
MS mass spectrum
Me methyl
MES maximal electroshock
NMR nuclear magnetic resonance
p.o. *per os*
^{i}Pr isopropyl
Pr propyl
RNA ribonucleic acid
SAR structure-activity relationships
SD standard deviation
SEM standard error of the mean
THF tetrahydrofuran
THP tetrahydropyranyloxy
TLC thin layer chromatography
UHP urea hydrogen peroxide

UTP uridine 5'-triphosphate

WHO World Health Organization

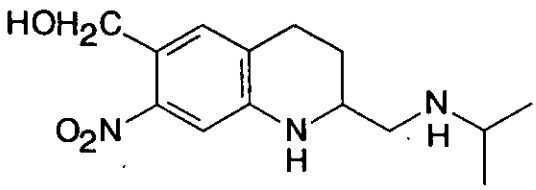
CONTENTS

CHAPTER ONE

GENERAL INTRODUCTION

1.1 BACKGROUND

Oxamniquine, 6-hydroxymethyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, (1), is a potent schistosomicide active principally against *Schistosoma mansoni*, the causative agent of the tropical disease, mansoni schistosomiasis commonly known as bilharzia.



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1.1.1 Schistosomiasis

Schistosomiasis, also known as bilharzia or snail fever, is a water-borne infection of man caused by a trematode worm, the schistosome, which has a water snail intermediate host. It is the most prevalent water-borne disease in rural areas in tropical countries and second only to malaria in its harmful social and economic effects.[1] At least 200 million people are thought to be infected.[2]

The symptoms of the disease depend on the parasite species. There are three species causing significant infection in man; *Schistosoma mansoni*, *Schistosoma haematobium* and *Schistosoma japonicum*. *Schistosoma mansoni* occurs extensively in Africa, the Middle East, South and Central America; *S.haematobium* in Africa and Middle East and *S. japonicum* in the Far East. There are two additional species which occur to a lesser extent: *S. intercalatum* is limited to Africa and *S. mekongi* to South East Asia.

(a) Life-cycles

Schistosomiasis is transmitted to man by contact with water habouring infected snails which act as the intermediate hosts in the parasite life cycle. Humans appear to be the primary host for *S. mansoni* and *S. haematobium* while domestic animals may serve as reservoir hosts for *S. japonicum*. Each schistosome species has its own snail species as intermediate host; generally *Biomphalaria* for *S. mansoni*, *Oncomelania* for *S. japonicum*, and *Bulinus* for *S. haematobium*. These snails release free-swimming larvae (*cecariae*), which rapidly penetrate the skin on contact. Immature parasites (schistosomula) then travel via the lymph and blood vessels to the lungs and liver. The development into small, mature parasitic

worms (schistosomes), occurs in the hepatic portal blood system prior to moving to species specific locations. The worms, about 1cm in length, exist as intertwined male and female worms, with the female held in the gynaecophoral canal of the male constantly being fertilized and laying eggs. The male worm drags the female through the vessels by attaching and releasing its ventral sucker. The worms migrate to and cling onto the inside walls of bladder or intestinal veins in the human host where they may reside for several years. The worms do not multiply but large numbers of tiny eggs are continually discharged by the female. Most of these eggs remain within the tissues especially the intestines, liver or the urinary tract. Some eggs are eventually excreted in the urine or faeces of the infected person and, on contact with fresh water, hatch to liberate larvae (*miracidia*) which infect the appropriate snail species and complete the life-cycle.(Figure 1.1)

(b) Pathology

All three species are obligate intravascular parasites and are not found in ducts, cavities or other tissues. The pathogenesis of schistosomiasis is correlated with extent of worm burden, the number eggs in the tissues and the extent of the inflammatory reaction to them. Each schistosome species produces characteristic inflammatory reactions caused by entrapped eggs in the tissues and organs in the body. The intestine, liver and spleen are affected by *S. mansoni* and *S. japonicum* resulting in intestinal schistosomiasis. Vesicular schistosomiasis results from the infection of the bladder and urinogenital system by *S. haematobium.* Tissue damage is progressive and continuous. After many, mainly asymptomatic years the progressive fibrotic tissue damage becomes evident. Other tissues, the lungs and CNS, may also be affected.
Schistosomiasis is a debilitating disease. Weakness and lethargy are the main complaints. However, in severe cases, the disease can be fatal. Definitive diagnosis is generally based on finding typical eggs in the urine or stools.

Specifically, infection due to *S. mansoni* is characterized by allergic skin reactions at the site of cercarial penetration plus cough, hepatitis, fever, malaise, abdominal pain and tenderness of the liver. Hepatosplenomegally and accummulation of ascitic fluid in the peritoneal cavity, giving the typical swollen abdomen, are characteristic of chronic *S. mansoni* infection. *Schistosoma mansoni* eggs are deposited primarily in the liver but may also appear in the lungs and other tissues. Egg deposition in the spinal cord or the brain leads to severe neurological disorders.

Diagnosis of *S. mansoni* infection is by demonstration of the eggs in the stool, rectal biopsy or by serological tests.

(c) Prevention and Control of Schistosomiasis

The main ways of preventing and controlling schistosomiasis include: education regarding the worm life cycle; health education and improved sanitation; interruption of the snail life cycle by snail eradication using molluscides or specifically killing of the parasite in the host by chemotherapy.

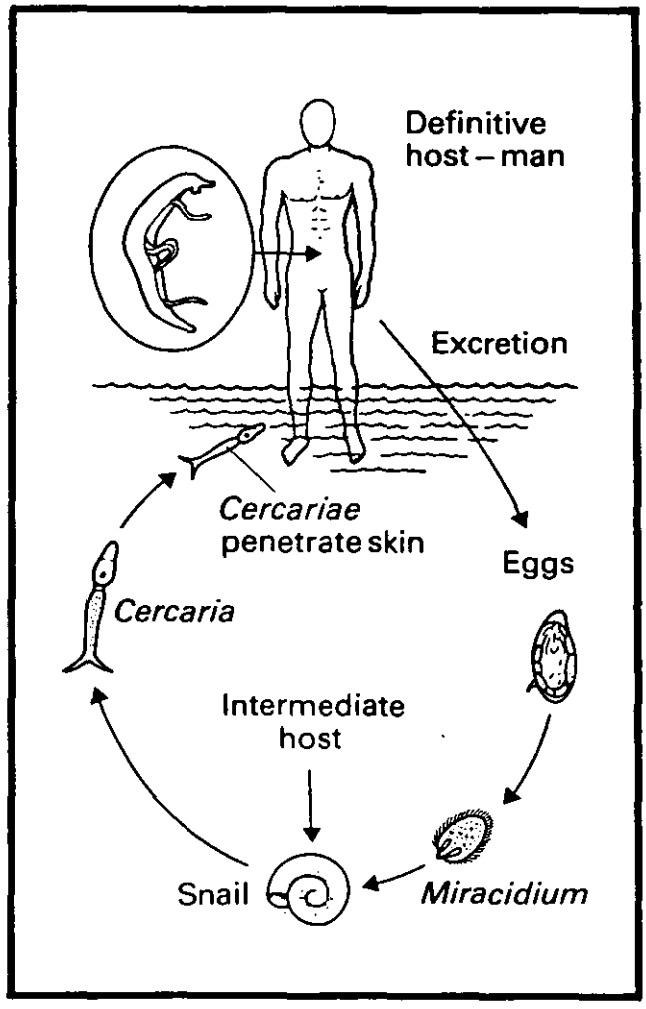


Figure 1.1. Life-cycle of the schistosome worm.[1]

1.1.2 The Chemotherapy of Schistosomiasis

The long term aims of chemotherapy are two fold: (i) to erradicate or to control the infection in the patient as an individual and (ii) in endemic areas, to benefit the community in which he lives. The immediate aim of chemotherapy is the control of morbidity caused by eggs deposited in tissues. An ideal chemotherapeutic agent for the control of schistosomiasis would be effective, non-toxic, cheap, safe and easily administered by non-professional personnel in non clinical situations in a single dose treatment.[3]

Antischistosomal drugs maybe classified simply according to their spectrum of antischistosomal activity.[4] This is summarized in Table 1.1.

Table 1.1 Antischistosomal Drugs

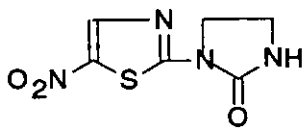
Drugs effective against one schistosome species	Oxamniquine (**1**) (Vansil®, Mansil®, Pfizer Inc.). *S. mansoni* Metrifonate (**4**) (Bilarcil® Bayer AG). *S. haematobium*
Drugs active against two schistosome species	Hycanthone mesylate (**3**) (Etrenol®, Winthrop). *S. mansoni, S .haematobium*
Drugs active against all schistosome species infecting man	Praziquantel (**6**) (Biltrcide®, Bayer AG). *S. japonicum, S. mansoni, S. haematobium, S. intercalatum, S. mekongi.* Niridazole (**2**) (Ambilhar®, Ciba-Geigy). *S. japonicum, S. mansoni, S. haematobium, S. intercalatum.* Trivalent antimonials (Stibocaptate, Astiban®, Roche). *S. japonicum, S. mansoni, S. haematobium, S. intercalatum.*
Drugs under development	Amoscanate (**8**) (C9333-Go/CGP450, Ciba-Geigy, also synthesized in the Peoples Republic of China). All common schistosomes infecting man . Oltipraz (**7**) (R.P. 35.972, Rhone-Poulenc). *S. mansoni, S. haematobium, S. intercalatum.*

(a) Individual Drugs

Trivalent antimony compounds

The use of trivalent antimony compounds in schistosomiasis began in 1918 with the advent of antimony potassium tartrate.[5] However, the inconvenience of parenteral administration, the variable efficacy of the drugs, the prolonged course of therapy and toxicities have limited the use of these drugs. There are few, if any, indications for the use of trivalent antimonials in the modern chemotherapy of schistosomiasis.

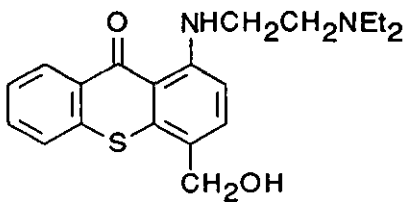
Niridazole [1-(5-Nitro-2-thiazolyl)-2-imidazolinone]



2

Niridazole (**2**), as the first metal-free drug to show activity against all three human schistosomes, occupied an important place in the history of the chemotherapy of schistosomiasis. However, its clinical use was associated with rarely encountered but serious side effects particularly neuropyschotic changes including visual and auditory hallucinations, convulsions and violent behaviour especially in *S. japonicum* and hepatosplenic *S. mansoni* cases.[6,7] Niridazole was later shown to be a powerful immunosuppressant and to be carcinogenic in rats, mice and hamsters. It has now been superceded by newer drugs.[6]

Hycanthone [1-(2-Diethylaminoethyl)-4-hydroxymethyl-thioxanthen-9-one]



3

Hycanthone (**3**) is highly effective in the treatment of *S. mansoni* and *S. haematobium* infections. It is effective after a single intramuscular dose of 2.5 to

to 3.5 mg/kg (of base). It is, however, regarded as a second line choice because of its known hepatotoxic, mutagenic and teratogenic potential.

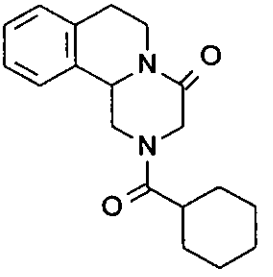
Metrifonate [O,O-Dimethyl (2,2,2-trichloro-1-hydroxyethyl) phosphonate]

$$(MeO)_2P(O)CH(OH)CCl_3 \longrightarrow (MeO)_2P(O)CH{=}CCl_2$$

4 **5**

The accidental discovery that an organophosphorus insecticide killed human schistosomes produced one of the cheapest and safest drugs. Metrifonate (**4**) is presently considered the drug of choice against *S. haematobium*.[7] Metrifonate is a potent anticholinesterse, its mode of antischistosomal activity is thought to be by blockade of cholinesterase enzymes, including acetylcholinesterase, resulting in higher than normal levels of acetylcholine and culminating in the incoordinate and sustained contraction of schistosome musculature and impaired mobility. The antischistosomal activity of metrifonate is mediated primarily through the metabolite dichlorvos (**5**) which is even more effective at blocking acetylcholinesterase systems in the schistosome. Metrifonate has a greater effect on the acetylcholinesterase systems of schistosomes than those of the mammalian hosts and the occurrence of cholinergic side effects during treatment is extremely low.

Praziquantel [2-(cyclohexylcarbonyl)-1,2,3,6,7,11b-hexahydro-4H-pyrazino (2,1-a)isoquinoline-4-one]

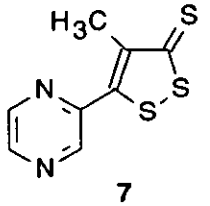


6

Praziquantel (**6**), a broad spectrum anthelminthic introduced for widespread clinical use in the early 1980's, is the current drug of choice for the treatment of all forms of schistosomiasis in man including hycanthone/oxamniquine resistant *S. mansoni*.[2] It has high therapeutic activity against all schistosome species pathogenic to man and, additionally, many other trematode and cestode infections. The exposure of schistosomes to therapeutic levels of praziquantel leads to immediate tetanic muscle contractions followed by rapid vacuolization of the tergument. Praziquantel is known to interfere with inorganic ion transport

mechanisms, decreasing potassium and increasing calcium and sodium ion flux, resulting in contraction of worm musculature. Vacuolisation of the schistosome tergument finally leads to the disruption of the tegumental layer. The exact mechanism whereby lethality is produced is not understood. Praziquantel is well tolerated with no reported toxic effects on vital organs, systems and functions. The side effects experienced, gastrointestinal disturbances, headache, dizziness, fever, fatigue and urticarial skin eruptions, are relatively mild and transient and rarely require additional treatment.[4]

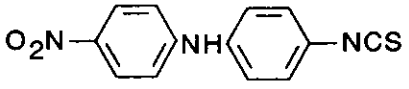
Oltipraz [4-Methyl-5-(2-pyrazinyl)-1,2-dithiole-3-thione]



7

Oltipraz (**7**) was one of the newer compounds to be developed as an antischistosomal agent. The drug was effective against *S. haematobium, S. mansoni* and *S. intercalatum.* In contrast to praziquantel, oltipraz was a very slow-acting drug; approximately two months were required before full schistosomicidal effects become manifest. However, clinical trials with oltipraz were suspended in 1984 due to late onset toxicity.

Amoscanate [4-Nitro-4'-isothiocyanatodiphenylamine]



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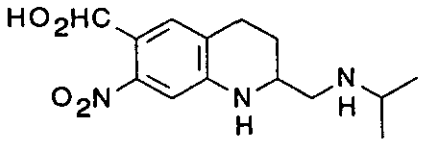
Amoscanate (**8**) is a broad spectrum anthelminthic effective against all the common schistosome species pathogenic to man as well as intestinal nematodes and filariae. Amoscanate is a yellow crystalline compound insoluble in water and most common organic solvents. Although the antischistosomal activity of amoscanate was reported in 1976,[6] this lack of solubility has hampered the development of proper dosage forms of the drug. The drug is undergoing clinical trials in man and is not yet available for widespread clinical use.[4]

At the present time only three of the drugs with established antischistosomal activity, metrifonate (**4**), oxamniquine (**1**) and praziquantel (**6**), are considered suitable for large-scale chemotherapy. Of these, praziquantel has transformed the treatment of schistosomiasis since it is effective, often in a single dose against all

schistosomes and is well tolerated[2] and oxamniquine is unique in that it affords high species specific activity against *S. mansoni* infections. There have, however, been no significant additions to the number of clinically useful antischistosomal drugs since the demise of oltipraz.

1.1.3 Oxamniquine



1

Oxamniquine is one of the preferred drugs for the treatment of all forms of *S. mansoni* infections in man where it achieves high parasitology cure rates.[1]

(a) Clinical Use

Oxamniquine (Vansil®, 250mg capsules) is indicated for all stages of *S. mansoni* infection including the acute phase and the chronic phase with hepatosplenic involvement. African strains of *S. mansoni* vary in their susceptibility to oxamniquine and are less susceptible than South American strains. Dosage is therefore dependent on the geographical origin of the infection. Total doses range from 15 to 60 mg per kg body-weight given over one to three days, preferably after food.[8] In children the dose may be divided and given over a 12 hour period. The treatment may have to be repeated every six months, should the patient continue to reside in an area where *S. mansoni* is endemic.

Many strains of *S. mansoni* exist with different susceptibilities to treatment with oxamniquine as shown by the different dosages required in different geographical areas.[8] The difference in strain response is not due to differing bioavailability of oxamniquine, since drug serum concentrations in patients from Brazil, South Africa and East Africa were very similar following equivalent doses, indicating that the variation is a property of the schistosome strain.[4] An hycanthone-resistant *S. mansoni* strain, that has been found to be invariably cross-resistant to oxamniquine, has been isolated from humans and laboratory animals.[9,10] Resistance to hycanthone (**3**) and oxamniquine was first reported in patients from Brazil and later confirmed by other investigators.[10,11] Oxamniquine-tolerant worms have recently been reported in Kenya.[12] *Schistosoma mansoni* strains which are resistant to oxamniquine and hycanthone but reponsive to praziquantel (**6**) have been reported in patients and praziquantel has been used to cure patients

who did not repond to oxamniquine.[10] Resistance to oxamniquine and hycanthone can be induced experimentally in mice and is genetically transmitted.[13]

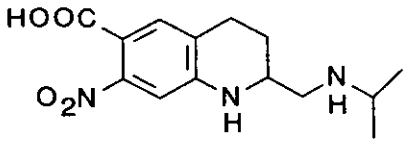
For patients with mixed infections of *S. mansoni* and *S. haematobium*, oxamniquine may be administered concurrently, safely and effectively with metrifonate (**4**).[14]

(b) Pharmacokinetics

Oxamniquine is well absorbed after oral or parenteral administration. Rapid oral absorption gives maximum serum concentrations 0.5 to 3 hours after dosage.[15] At the time of peak serum concentrations only 10 per cent of drug related material is comprised of unchanged drug. The drug is subject to presystemic metabolism. Animal studies[16] have shown that oxamniquine is extensively metabolized to the 6-carboxy derivative by enzymes in the gut wall before or during systemic absorption. Individual variation in presystemic metabolism may account for the individual variation in the serum concentration of oxamniquine and the time to peak, 2.28 mg/l SD 1.35 and 3 hours SD 1.4 respectively, observed in human pharmacokinetic studies.[17]

In vivo studies using female Wistar rats have shown that oxamniquine readily passes into rat brain tissue following intravenous administration of a 15 mg/kg dose.[18] Maximum brain concentrations of oxamniquine were achieved one hour after dosing and at all sampling times oxamniquine levels were higher in brain tissues compared to the corresponding plasma samples. The hydrophobicity parameter, Log P (a measure of the lipohilicity of a compound) for oxamniquine has been experimentally determined as 2.245, S.D 0.064.[19] This is in the range of the optimum Log P exhibited by compounds previously shown[20] to be capable of penetrating into the CNS . These compounds have an optimal Log P value of 2.0.

Studies in man and animals[21] showed that oxamniquine was extensively metabolized to inactive acidic metabolites which were excreted in the urine. In human volunteers only 0.4 to 0.9 percent of the oral dose was excreted as unchanged drug in the urine. Two principle metabolites, arising from two sites of biotransformation, were isolated.



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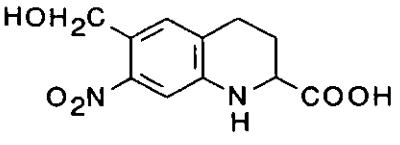


10

The major metabolite (**9**) arises from the oxidation of the 6-hydroxymethyl group to a carboxylic acid. In man this constitutes 41 to 73 percent of the metabolites excreted in the urine. The 2-carboxylic acid derivative (**10**), arising from oxidative cleavage of the side chain, is excreted only in trace amounts in man. Oxamniquine is rapidly cleared with most of the metabolites excreted in the first twelve hours after dosage. No drug is detectable in the twenty-four hour serum samples.

The acid metabolite (**9**) and the methyl ester of the metabolite (**10**) have no schistosomicidal activity.[22]

(c) Pharmacological Effects

(i) Schistosomes

Oxamniquine has specific spectrum of activity against *S. mansoni.* It is ineffective against the other major schistosome species: *S. japonicum* and *S. haematobium.*

Oxamniquine stimulates motor activity in schistosomes *in vitro* at relatively low concentrations, 10^{-4} to 10^{-5}M, while high concentrations, 10^{-3}M, paralyse the worm.[23] The onset of the responses is rapid. In isolated schistosomes oxamniquine increased the tonus and the frequency and amplitude of *S. mansoni* spontaneous contractions.[24] Both these effects are thought to involve a tryptaminergic mechanism, since they were blocked by the tryptaminergic agent, bromolysergic acid diethylamide. In addition both effects are calcium dependent; they are absent in calcium-free media and completely abolished by the Ca^{2+} channel antagonist, verapamil.

In vitro exposure of paired male and female worms to oxamniquine (12.0 μg/ml) resulted in immediate hyperactivity leading to unpairing of the worms.[25] Oxamniquine induces shift of both male and female worms from the mesenteric veins to the liver of the host.[26] The onset of the hepatic shift is relatively slow and is not complete until 6 to 7 days after a single dose of the drug. Male worms are more susceptible than female worms. While the shift of male worms is

irreversible, surviving unpaired female worms, may return to the mesenteric veins but egg production is permanently impaired.

The amount of drug related material in the males is four times greater than in the females, this parallels their greater sensitivity to oxamniquine.[25] However, only about 10% and 30% of the total drug present in males and females respectively is assayed as unchanged oxamniquine. Thus the major proportion of drug-related material present in both sexes is metabolites.[25] These metabolites have, however, been reported not to possess any schistosomicidal activity.[16]

The in *vivo* effects of oxamniquine on the tergumental surface of adult *S. mansoni* have been studied.[27] Pronounced tergumental alterations were observed in all worms. The amount of damage was more marked in males than females and was irreversible; all worms were eventually eliminated. Damage to the tergument exposes the worm to damage by the host, this is probably immunological.[27] Susceptibilty of schistosome strains to oxamniquine depends on the geographical distribution. African strains of *S. mansoni* differ in their susceptibility to oxamniquine but are generally less sensitive than the South American strains.[8]

Oxamniquine is unique in that it is effective against all stages of *S. mansoni.* It is the only agent active against the early acute stage. Other schistosomicides are only active during the first few days after exposure and when worms reach maturity.

(ii) Mammals

Oxamniquine injected intramuscularly in rats prolonged barbiturate hypnosis.[28] Oxamniquine has since been shown to inhibit antipyrene metabolism, indicating that it is capable of inhibiting liver enzymes. Metabolic inhibition, not direct CNS depression, is therefore the most likely cause of prolonged barbiturate hypnosis.

The neuromuscular blocking effects of oxamniquine, in doses exceeding therapeutic drug levels, have been demonstrated *in vitro.*[29] The effects of oxamniquine on the autonomic nervous system have also been demonstrated. Oxamniquine depressed or abolished electically-evoked contractions of the isolated chick oesophagus and isolated vas deferens muscle preparations suggesting blockade of parasympathetic and sympathetic transmissions respectively.[30]

Calcium dependent rhythmic and tonic contractions have been demonstrated in the chick isolated oesophagus, a smooth muscle preparation.[31]

The pharmacological effects of oxamniquine metabolites, the acids (**9**) and (**10**), in mammals have not been reported.

(d) Toxicity and Side Effects

For a compound with a relatively high potency of biological activity in one area, oxamniquine showed relatively low toxicity in most mammalian systems.[32]

(i) Animal Studies

Oxamniquine displayed low acute toxicity in mice, hamsters and rabbits. Acute hepatotoxicity was, however, observed in rats. The female rat was more susceptible than the male rat. [32,33]

Neurological disturbances of short duration were observed in beagle dogs after repeated oral doses.[32] No morphological lesion was found to correlate with the neurological disorder and between treatment periods the dogs appeared normal.

Chronic toxicity studies showed no evidence of carcinogenicity, mutagenicity or teratogenicity potential.[32] Although some aromatic nitro compounds maybe carcinogenic via formation of an aromatic amine[32] or hydroxylamino group[34], the reduction of the nitro group of oxamniquine to form an aromatic primary amine is not a significant metabolic pathway in mammals.[34]

(ii) Clinical Use

Oxamniquine, at all dosages, is generally well tolerated by patients. It commonly illicits acute, transientory, mild to moderate CNS effects manifested by dizziness, nausea, headache or drowsiness.[35,36] A modest post-treatment rise in temperature has been reported occasionally from several areas. Fever is a common side effect in Egypt where it appears to be characteristic, the cause is unknown but it is associated with increased immune complexes and excretion of antigens.[37]

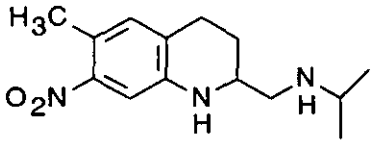
Severe CNS disturbances have, however, been reported in some individuals.[38] Transient encephalographic disturbances and seizures, as well as visual and auditory hallucinations, have been recorded.[39,40,41,42] These neuropsychiatric manifestations indicate that oxamniquine can affect the CNS and in rare cases severe reactions may result. Care should be taken in the treatment of patients with a history of convulsive disorders and it is considered prudent to administer anticonvulsant drugs prior to initiating oxamniquine therapy in patients with such history.[8]

Oxamniquine has been shown to partition into brain tissue to give brain levels greater than plasma drugs levels after intravenous administration to rats.[18] It has been suggested that the adverse neurological effects observed in some patients, after treatment with oxamniquine, may be due to sufficiently large quantities of the drug in the CNS.[18] Nevertheless, these effects have not limited the use of oxamniquine which has been extensively used for the field treatment of several million people in rural communities.

(e) Mechanism of Action

The exact mechanism of the schistosomicidal effect of oxamniquine is not fully understood. It is, however, thought to be multifactoral.

Oxamniquine has similarities to hycanthone (**3**).[43] Structurally oxamniquine and hycanthone contain an alkylaminoethyl amino side chain para to a critical hydroxymethyl group. Both are oxidative metabolites of the parent compounds UK 3883 (**11**) and lucanthone (**12**) respectively. Complete cross-resistance between oxamniquine and hycanthone, in strains of *S. mansoni* resistant to either drug has been demonstrated.[43] These chemical and biological similarities suggest similar mode of action for the two compounds .



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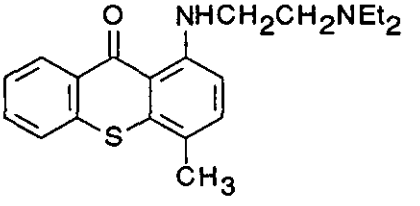


12

Two mechanisms, one a cholinergic mechanism and the second a mechanism involving alkylation of sensitive schistosome macromolecules, have been proposed.

(i) Cholinergic mechanism

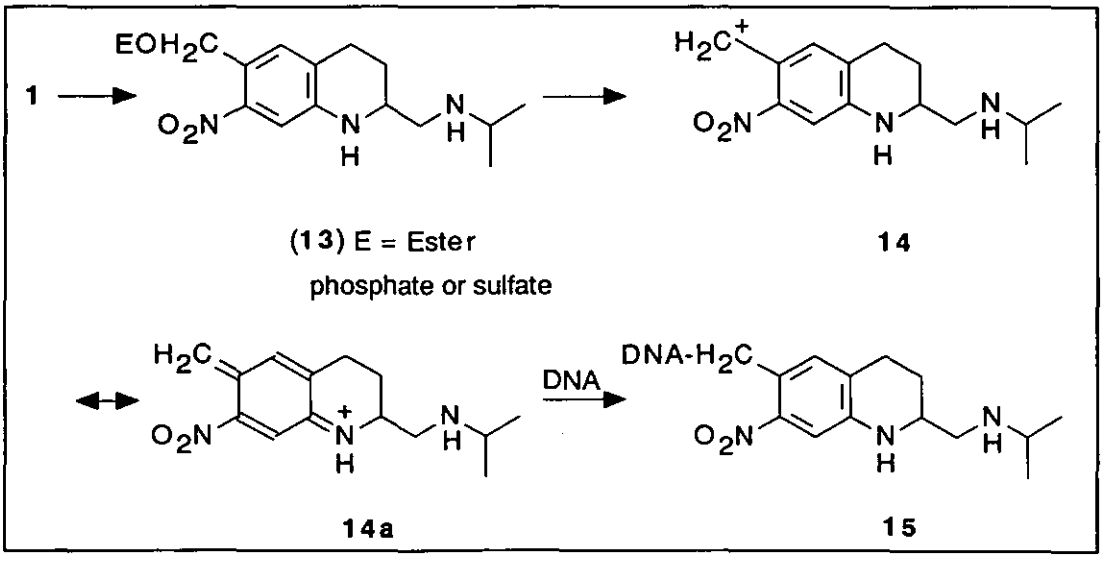
A cholinergic mechanism is thought to be responsible for the motor activity induced by oxamniquine, in susceptible schistosomes, in *vitro*.[44] Oxamniquine concentrations of 10^{-4} to 10^{-5}M stimulate motor activity while higher concentrations, 10^{-3}M, cause paralysis of the worms.

It is generally believed that, in schistosomes, serotonin functions as an excitatory neurotransmitter causing increased motor activity. Acetylcholine is thought to act

as an inhibitory neurotransmitter causing flaccid paralysis of the worms. It was proposed that the antischistosomal activity of oxamniquine, like that of hycanthone, was due to blockade of worm acetylcholine receptors, causing derangement of peristalsis, failure to feed and slow death by starvation.[45] However, observations contradicting this mode of action have showed a lack of correlation between schistosomicidal and anticholinergic properties of both oxamniquine and hycanthone.[46] Although oxamniquine caused increased motor activity in sensitive *S. mansoni*, the same effect was present in hycanthone-resistant *S. mansoni*. Motor activity, following essentially the same pattern, was also observed in *S. japonicum* which is refractory to both oxamniquine and hycanthone. Two precursors of oxamniquine and hycanthone, UK 3883 (**11**) and lucanthone (**12**) respectively, which are totally devoid of antischistosomal activity *in vitro,* displayed unequivocal anticholinergic effects on schistosomes when tested *in vitro*. These two precursors also influenced the motor activity of both hycanthone-sensitive and hycanthone-resistant *S. mansoni* and also *S. japonicum*. These observations suggest that the cholinergic mechanism does not account for the antischistosomal activity of oxamniquine.

(ii) Alkylation of schistosome macromolecules

A mechanism involving alkylation of sensitive schistosome macromolecules has been proposed.[47] The mechanism is thought to involve esterification of oxamniquine to the reactive ester (**13**) which spontaneously dissociates to give the ion (**14** - **14a**) which can alkylate DNA[48]. This mode of action is illustrated in Scheme 1.1 below.



Scheme 1.1 The proposed mode of action of oxamniquine.[48]

Oxamniquine has been shown to inhibit the incorporation of triturated uridine into hycanthone/oxamniquine-sensitive *S. mansoni.*[49] This demonstrates inhibition of RNA biosynthesis in the schistosome. A good correlation was obtained between schistosomicidal activity of the hycanthone/oxamniquine family and early irreversible inhibition of schistosome-uridine incorporation.[49] Using isolated hamster liver nuclei, oxamniquine was shown to irreversibly inhibit RNA biosynthesis in a mammalian system *in vitro.*[50] This irreversible inhibition suggests extremely tight binding to or a covalent modification of some component of the RNA biosynthesis machinery. These observations are in agreement with the suggestion that oxamniquine forms a DNA-drug adduct on metabolic activation.[48]

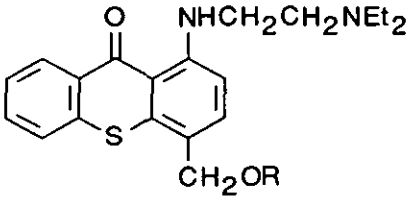
In vitro experiments have shown that oxamniquine binds to schistosome DNA.[51] Triturated oxamniquine was shown to bind to the DNA fraction of hycanthone-sensitive schistosomes, approximately one drug molecule per 50,000 base pairs. This is comparable to that found in mammalian cells after exposure to potent alkylating agents. No oxamniquine was associated with the DNA of hycanthone-resistant worms.

The evidence available to date supports the mechanism involving alkylation of sensitive schistosome macromolecules as the mode of action of oxamniquine. It is possible that extensive alkylation may interfere with DNA replication and RNA biosynthesis, this may eventually lead to worm death.[47]

It has been suggested that resistant schistosomes escape alkylation and death because they lack some activity which is necessary to effect conversion of oxamniquine to an alkylating agent.[51] In the case of hycanthone this mechanism was supported by the finding that hycanthone esters, the N-methyl carbamate (**16**) and the acetate (**17**) were active not only in sensitive worms but also in resistant schistosomes.[52,53] However, in the case of oxamniquine, the N-methyl carbamate (**18**) and the acetate ester (**19**) failed to show any activity against resistant schistosomes.[49] Thus, although the suggestion that oxamniquine alkylates schistosomes has been confirmed, the hypothesis that resistance is due to a lack of the esterifying activity failed to account for all the experimental observations.

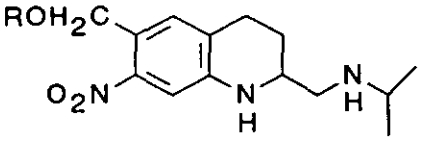
It should be noted, however, that the concentrations required to alkylate sensitive schistosomes *in vitro* are much higher than those attained after therapeutic dosage. This suggests that the schistosomicidal activity is multifactoral and/or the role of in *vivo* metabolism transforming the drug into a more potent metabolite.[50]

It is important to note that although oxamniquine, like hycanthone, binds to DNA, it lacks mutagenic properties because it is not an intercalating agent. Oxamniquine did not modify the DNA melting temperature,[51] used as an indication of the intercalating potential of a compound.



R

(O)CNHCH$_3$ **(16)**
(O)CCH$_3$ **(17)**



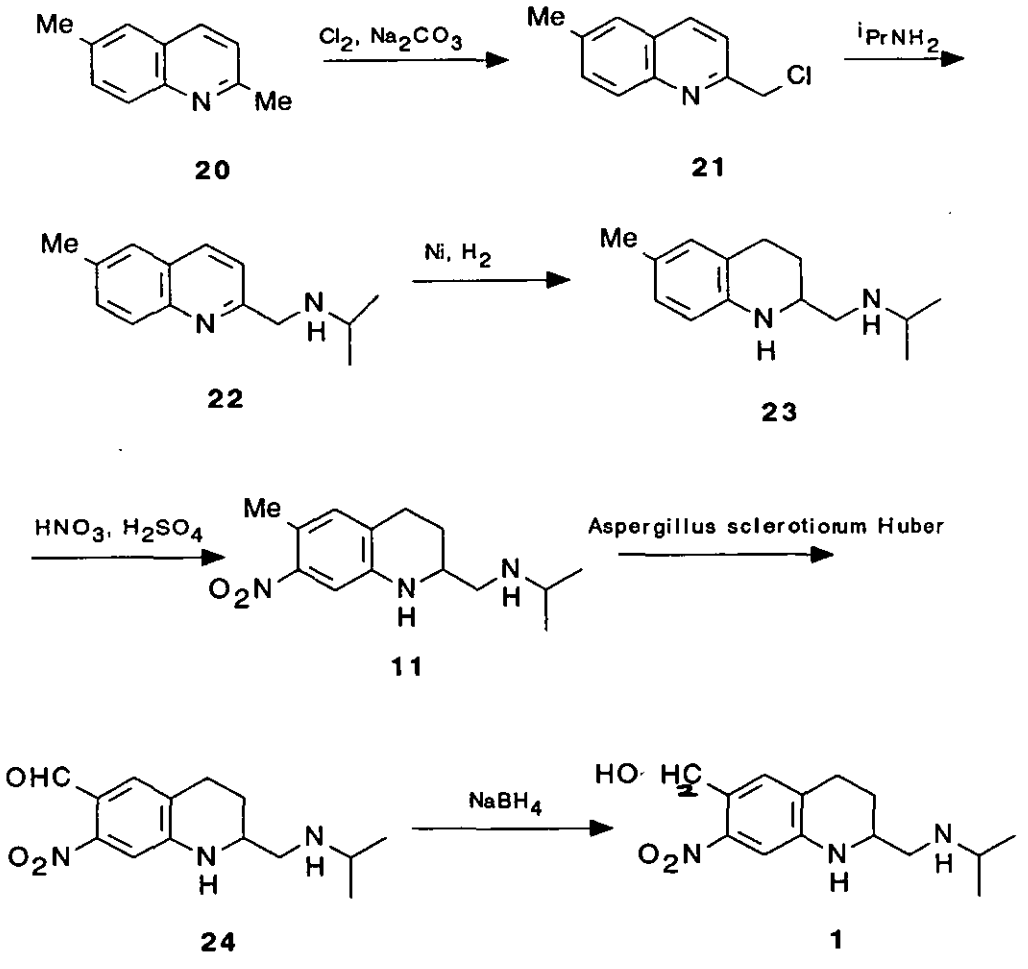
R

(O)CNHCH$_3$ **(18)**
(O)CCH$_3$ **(19)**

(f) Chemistry

(i) Synthesis

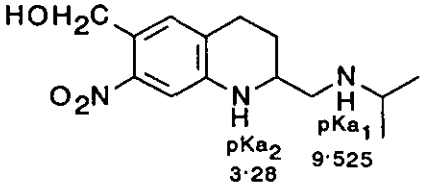
Oxamniquine (**1**), the oxidative metabolite of UK 3883 (**11**), was first synthesized by Richards,[54] as shown in Scheme 1.2.



Scheme 1.2. The synthesis of oxamniquine

(ii) Physico-chemical properties

Oxamniquine is a stable yellow powder, melting point (isopropanol) 147-149°C[54], only slightly soluble in water 0.03% w/v 27°C.[55] The pKa of oxamniquine are pKa_1 9.53 for the side chain nitrogen and pKa_2 3.28 for the ring nitrogen.[19] pKa_1 indicates that oxamniquine is strongly basic. Oxamniquine is 99.9% ionised at physiological pH, pH 7.4. The experimentally determined hydrophobicity parameter, Log P (octanol/water), for the neutral species has the value 2.245, S.D. 0.064.[19]



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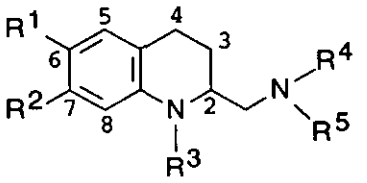
(iii) Stucture-Activity Relationships (SAR)

Oxamniquine belongs to a series of tetrahydroquinolines (**25**) containing an aromatic methyl adjacent to an electronegative substituent and para to an aminoethylamino residue.[56] The structural features essential for schistosomicidal activity in the tetrahydroquinoline class, as assessed by a primary mouse screen and secondary mouse screen, are shown in Figure 1.2.[57]



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	Active compounds	Inactive compounds
R^1	Me	H, Et
R^2	Electronegative substituent, order of decreasing activity NO_2 > CN > F > Cl > Br	H
R^3	H, Me, Et	Higher alkyl, acyl
R^4	H, lower alkyl (up to C_4)	Acyl, higher alkyl
R^5	Lower alkyl (up to C_4)	Acyl, higher alkyl

Figure 1.2 SAR of Oxamniquine

The features essential for activity are :-

i) an aromatic methyl group (R^1) adjacent to an electronegative substituent (R^2) and para to an aminoethyl group,

ii) both nitrogen atoms should be basic, non acylated and

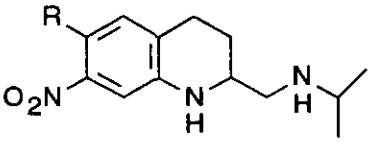
iii) for the terminal nitrogen; the isopropyl-NH showed the maximum activity.

Optical isomerism arises from the chiral centre at C_2. All compounds were tested as racemic mixtures. The dextrorotatory isomer is, however, the more active isomer.

UK 3883 (**11**) was the most active of the tetrahydroquinolines *in vivo* but was inactive *in vitro*. Metabolic studies confirmed that the 6-methyl group was converted to hydroxymethyl in several animal species. Oxamniquine is the active metabolite of UK 3883 and is active both *in vivo* and *in vitro*.

(iv) Structural Variants of Oxamniquine

Various tetrahydroquinoline derivatives have been synthesized and screened for their activity against murine schistosomiasis in the search for a potent schistosomicide. Those structural variants involving modifications at C_6 with retention of the N-isopropylaminomethyl side chain at C_2 and the 7-NO_2 are shown in Figure 1.3.



R	Activity against murine schistosomiasis
CH_3[a] (**11**)	very high activity monkey curative dose:- 50 mg/kg[b]
CHO[c] (**24**)	not documented
CH_2OH[a] (**1**)	very high activity monkey curative dose:- 15 - 20 mg/kg[b]
CH_2OR^1, (R^1 = 1 - 6C)[a] *e.g.* R^1 = Et (**26**)	very high activity
$CH_2OC(O)R^1$, (R^1 = 1 - 6C)[d]	not documented

[a] H. C. Richards, USP 3 821 228/1974.

[b] H. C. Richards, in The Role of Organic Chemistry in Drug Research, ed. S. M. Roberts and B. J. Price, Academic Press, 1985, 271 - 289.

[c] H.C. Richards, USP 3 929 784/1975.

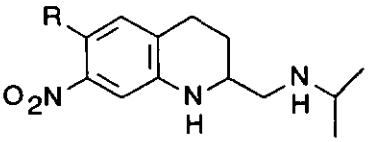
[d] H. C. Richards, Offenlegungsschrift 2 221 669/1972.

Figure 1.3 Structural variants of oxamniquine reported in the literature.

Oxamniquine was found to exhibit maximal antischistosomal activity *in vitro* and *in vivo* and was selected as the lead compound for development.

Other structural variations at C_6 of oxamniquine reported in the literature include the N-methylcarbamate (**18**) and acetate (**19**) esters as well as arylsulfonohydrazones of oxamniquine aldehyde *e.g.* (**27**).[49] These compounds were synthesized as model activated esters in an attempt to verify the proposed mechanism of action of oxamniquine, which is thought to be mediated via enzymatic activation of oxamniquine to a reactive ester which alkylates schistosome DNA[48], as shown in Scheme 1.1. However, in contrast to the preformed carbamate ester of hycanthone (**16**), which was shown to be active against both hycanthone/oxamniquine-sensitive and hycanthone/oxamniquine-resistant schistosomes *in vitro* as well as *in vivo*,[56] the oxamniquine esters (**18**)

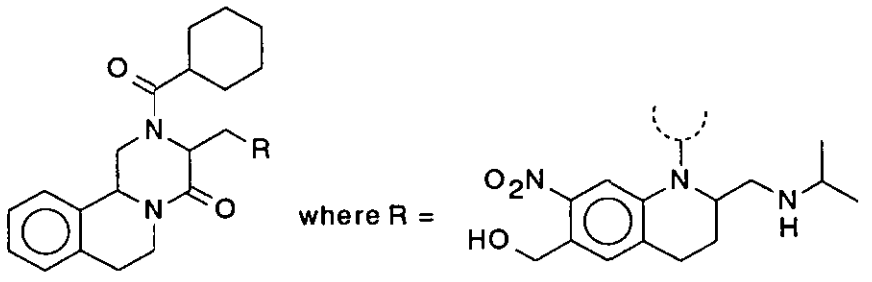
and (**19**) were shown to be active against the hycanthone/oxamniquine sensitive worms only.[49] No activity was displayed against hycanthone/oxamniquine resistant worms.



R = 2,4,6,-$(CH_3)C_6H_2SO_2NHN{=}CH$ (**27**)

The synthesis of an oxamniquine-praziquantel adduct (**28**) has also been reported.[58] This was shown to be active in both hycanthone/oxamniquine sensitive and resistant schistosomes and exhibited higher schistosomicidal activity than the individual drugs when used on their own.

The synergistic effect of oxamniquine administered simultaneously with praziquantel was initially demonstrated in mice infected with *S. mansoni*[59] and subsequently confirmed in clinical trials.[60] Efficacy was produced at single doses lower than for either drug alone. The effect was synergistic against *S. haematobium* as well as *S. mansoni.* The combined treatment was well tolerated, side effects being of low incidence and self limiting. It has been suggested that the low dose co-administration could confer a cost advantage with the potential benefit of an increase in tolerance without loss of efficacy.[60] As therapy with both drugs concomittantly would be more convenient with a combination tablet, a recent study indicated that oxamniquine and praziquantel did not interact with each, or with several common formulation excipients, in the solid state.[61] On the basis of these results, it was concluded that a combination tablet dosage form of the two drugs was feasible.[61]



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1.2 RATIONALE AND JUSTIFICATION

The discovery of oxamniquine represented a significant break-through in the treatment of schistosomiasis. It is efficacious in a single oral dose, relatively well tolerated as compared with other schistosomicides, effective against all stages of *S. mansoni* and safe for use in patients of any age and at any stage of the disease.[62] Although praziquantel is now considered the drug of choice for the treatment of all infections due to *S. mansoni*, *S. haematobium* and *S. japonicum*, oxamniquine is included in the WHO List of Essential Drugs and is still widely used for the field treatment of schistosomiasis mansoni. The central effects of oxamniquine are therefore of clinical importance and there is a need to establish the mechanism by which they arise so that effective methods of minimizing them can be developed.

Little is known about the pharmacology of oxamniquine in man. The central effects may be due to oxamniquine and/or its metabolites and may/may not be related to the schistosomicidal action of the drug. Since oxamniquine is rapidly metabolized *in vivo* to the 6-carboxylic acid derivative (**9**) which is biologically inactive, it seemed appropriate to investigate the effect of structural modification at C_6 on the biological activity and CNS effects of oxamniquine.

It was therefore proposed to synthesize analogues of oxamniquine by structural modification at C_6 to produce analogues which:-

(i) would retain the critical hydroxy functionality required for biological activity and therefore retain schistosomicidal activity,

(ii) would have different metabolic pathways and therefore not produce the 6-carboxylic acid metabolite, and

(iii) may/ may not have CNS effects.

1.3 OBJECTIVES

We proposed the hypothesis that structural modification of oxamniquine at C_6 would result in structural analogues with :-

(i) oxamniquine-like or other activity,

(ii) increased biological activity, and

(iii) decreased toxicity, especially CNS effects.

The specific aims of the project were firstly to synthesize structural variants of oxamniquine by chemical modification at C_6 and secondly to investigate the effect of structural modification on the biological activity, in particular any CNS activity, and the mode of action.

CHAPTER TWO

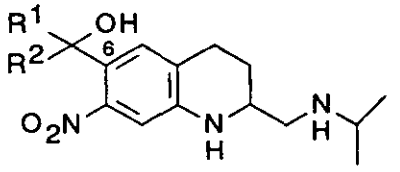
PREPARATION OF OXAMNIQUINE ANALOGUES

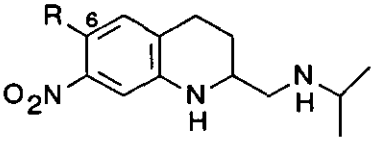
2.1. INTRODUCTION

The aim of this work was to prepare structural variants of the schistosomicide oxamniquine (1) which would be used in studies to determine the effect of the structural modification on biological activity, particularly the CNS activity. It was proposed to synthesize a series of oxamniquine analogues for use in preliminary structure activity relationship studies.

As alluded to in Chapter One, the mode of action of oxamniquine is thought to involve enzymic activation of oxamniquine, in sensitive schistosomes, to give a reactive ester that acts as an alkylating agent and binds covalently to schistosomal DNA.[48] A schematic representation of the mode of action is given in Scheme 1.1. Extensive alkylation of schistosome DNA may interfere with DNA replication and RNA biosynthesis resulting in worm death. The presence of a good leaving group at the critical C_6 position is essential for formation of the activated ester or other reactive intermediate which spontaneously dissociates, *in vivo*, to give the alkylating species. We therefore set out to investigate the effects of changes in the chain length and size of the C_6 substituent as well as the substitution of other groups at this position on the biological activity.

2.1.1. Synthetic Targets





R^1	R^2		R
H	H	oxamniquine (**1**)	CO_2H acid metabolite (**9**)
Me	H	secondary alcohol (**29**)	CH_2CH_2OH (**32**)
Me	Me	tertiary alcohol (**30**)	CH_2CO_2H (**33**)
H	CH_2OH	diol (**31**)	$CHCH_2O$ (**34**)
			CH_2Br (**35**)
			CH_2NHR (**36**)

Fig. 2.1 Oxamniquine analogues proposed as the synthetic targets

Two groups of target compounds were envisaged. The Fig. 2.1 shows the oxamniquine analogues which were proposed as the synthetic targets of the project.

The first series of compounds, represented by the alcohol homologues of oxamniquine (**29**) and (**30**), retain the hydroxy functionality critical for schistosomicidal activity but being secondary and tertiary alcohols respectively would not be oxidatively metabolized to give the 6-carboxylic acid metabolite (**9**). In fact successive substitution of the carbon with methyl groups would increase the steric bulk of the functionality and sterically hinder the hydroxyl group to metabolic processes. It was of importance to assess the effect of chain branching on biological activity as would be illustrated by the secondary and tertiary alcohol series of compounds (**29**) and (**30**) respectively.

The carboxylic acid (**9**), the major metabolite of oxamniquine, is present in high levels in blood plasma of patients treated with the drug but has no schistosomicidal activity. It was important to synthesize and evaluate the CNS activity of this metabolite (**9**) in order to determine its contribution to the central effects of the parent molecule.

The diol (**31**) was considered an interesting compound to synthesize and compare with oxamniquine as it represents a one carbon extension of oxamniquine containing two hydroxy functionalities.

The second series of oxamniquine analogues proposed were compounds in which the 6-hydroxymethyl group of oxamniquine is replaced by other substituent groups represented by R. The one carbon alcohol homologue of oxamniquine (**32**) and its corresponding carboxylic acid (**33**) were considered as representative examples for the investigation of the effect of one carbon homologation on biological activity.

As DNA alkylation is thought to be one of the mechanisms for the schistosomicidal effect of oxamniquine,[48] it was anticipated that the epoxide (**34**) would serve as a prototype in the study of the effect of structural modification on biological activity. The epoxide (**34**) would be a reactive intermediate and a potent DNA alkylator in its own right without requiring bioactivation. The synthesis and biological screening of this epoxide was therefore envisaged as central to the understanding of the biological action of oxamniquine. It was expected that it would be possible to demonstrate alkylation of non schistosomal DNA by this activated oxamniquine species *in vitro*. Previous studies have failed to demonstrate the binding of oxamniquine to calf thymus DNA using flow dialysis method[50], an *in vitro* laboratory model of DNA binding. Oxamniquine binding to DNA has only been demonstrated in the schistosome worm.[51] The activated esters of oxamniquine synthesized by El Hamouly[49] to verify the

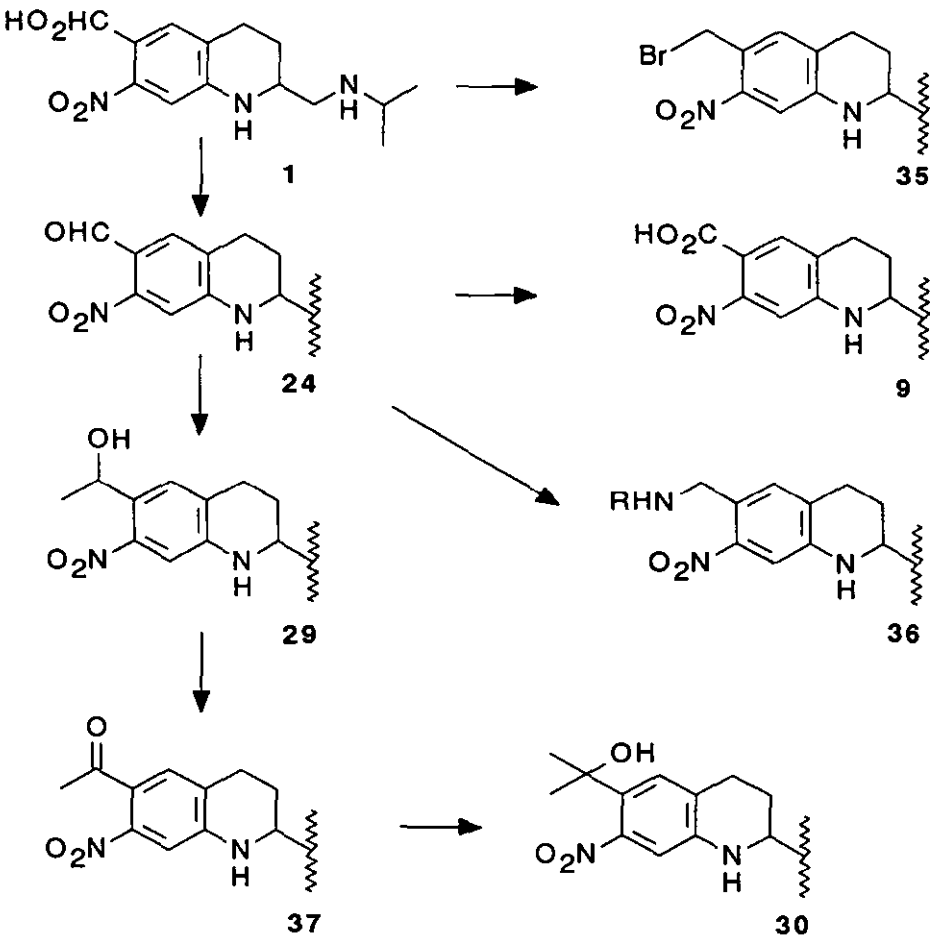
proposal that the mechanism of oxamniquine is mediated by an activated species and that resistance to oxamniquine is due to the absence of this esterifying enzyme in the resistant schistosome, failed to demonstrate schistosomicidal activity in hycanthone-oxamniquine resistant worms. This suggested that, unlike hycanthone, resistance to oxamniquine is not due to the absence of the esterifying enzyme.
It was also anticipated that this epoxide (**34**) would provide a versatile synthetic intermediate readily amenable to functional group transposition and affording access to other oxamniquine analogues.

In addition it was proposed to investigate the effect of alternative leaving groups on the biological activity. Replacement of the 6-hydroxymethyl functionality with a bromomethyl group was expected to provide the bromide (**35**), containing a good leaving group which would be readily converted, *in vivo*, to a reactive alkylating species with the potential to alkylate DNA and therefore have possible schistosomicidal activity.

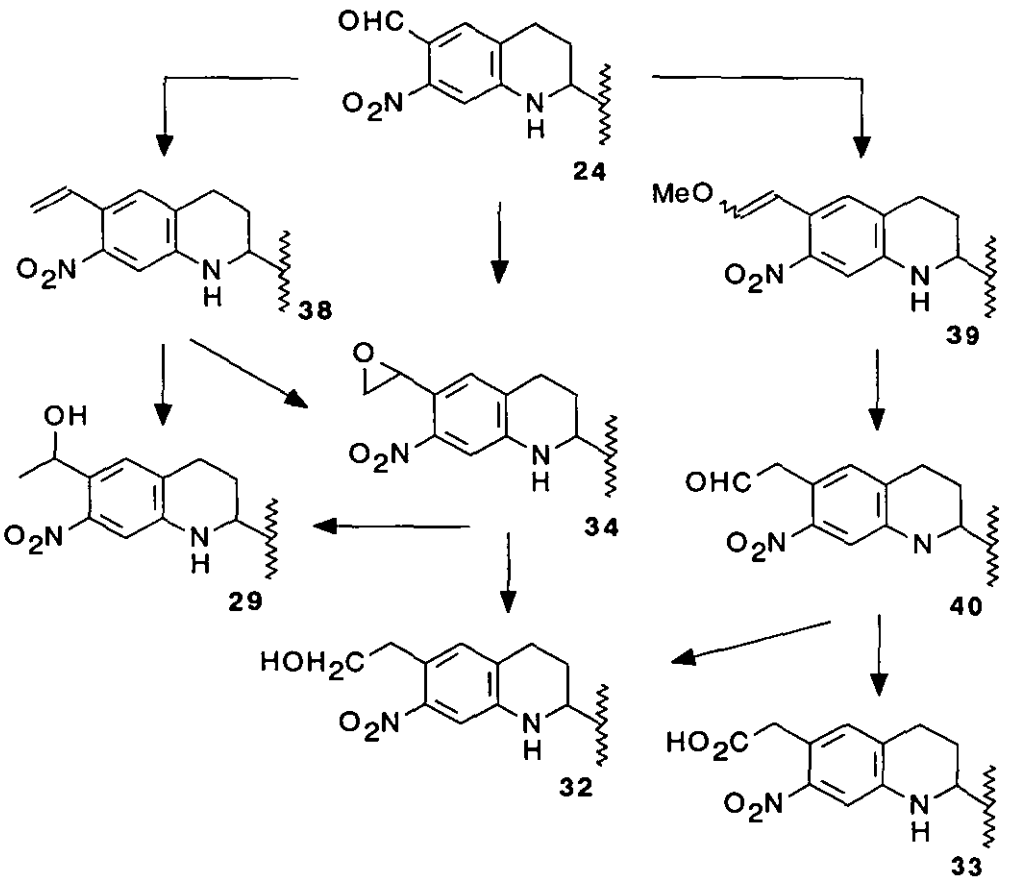
Bioisosteric replacement, the substitution of atoms or group of atoms for others in the parent molecule to provide compounds with broadly similar biological properties with a diversity of structure, has played a fundamental role in drug development covering a wide range of important pharmacological classes. The amine (**36**) represents the entry into a bioisosteric series in which the oxygen functionality is replaced with its NH isostere.

2.1.2. Synthetic Strategy

The strategy proposed for the synthesis of oxamniquine analogues is presented in Schemes 2.1 and 2.2.



Scheme 2.1 Synthetic strategy 1

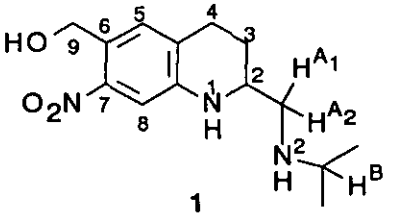


Scheme 2.2 Synthetic strategy 2

2.2. RESULTS AND DISCUSSION

2.2.1. 6-Hydroxymethyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine (1)

Oxamniquine reference compound, UK 4271 Lot 603/OX/701, was kindly donated by Pfizer Central Research, Sandwich, Kent, England. Analysis of the IR, proton-NMR and mass spectra provided full characterization and reference data with which to compare the structural analogues subsequently synthesized. Spectral assignments of the proton NMR spectrum are presented in Table 2.1.



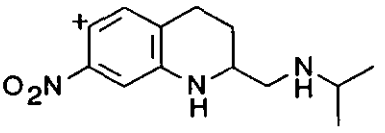
1

Chemical shift, ppm	Appearance	Proton
1.06-1.10	6H, 2 doublets, J 5.5 Hz	$CHMe_2$
1.47-1.63	3H, broad multiplet	N^2-H, OH, 3-H
1.92-2.01	1H, multiplet,	3-H
2.42-2.50	1H, double doublet, J 10.2 and 3.4 Hz	A_2
2.75-2.83	3H, multiplet	4-CH_2, B
2.86-2.92	1H, double doublet, J 6.8 and 3.4 Hz	A_1
3.23-3.32	1H, multiplet	2-H
4.69	2H, singlet	9-CH_2
5.06	1H, singlet	1-H
7.11	1H, singlet	5-H
7.20	1H, singlet	8-H

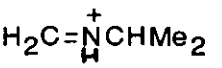
Table 2.1 Proton NMR chemical shift assignments for oxamniquine

The appearance of of the methyl protons of the isopropyl substituent at N^2 as a pair of doublets is due to the diastereotopic nature of these protons. Spectral assignments of the proton NMR spectrum of oxamniquine has recently been reported in the literature.[63]

The chemical ionization mass spectrum gave the measured mass 280.1661 for the M+H peak from the molecular formula $C_{14}H_{21}N_3O_3$. The major fragments detected were m/z 248 and 72 which may be assigned as the fragments (**41**) and (**42**) respectively.



41

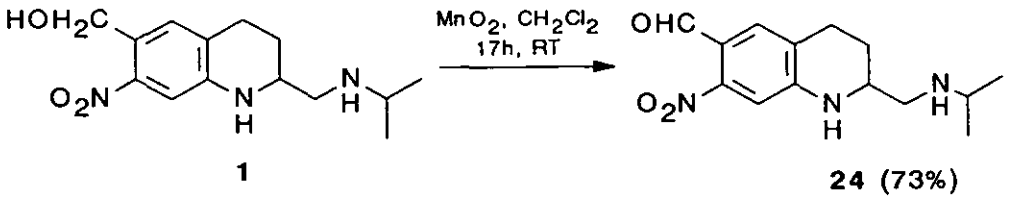


42

2.2.2. 6-Methanoyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetra-hydroquinoline, Oxamniquine aldehyde (24)

The aldehyde (**24**) was required as the synthetic precursor for the oxamniquine analogues: the alcohols (**29**), (**30**), and (**32**), the acid (**9**), the epoxide (**34**) and the arylamine (**36**) as shown in Schemes 2.1 and 2.2. Oxidation of oxamniquine (**1**) to the aldehyde was therefore considered the first step in the functionalisation of the molecule at position 6. The aldehyde (**24**) provides a reactive intermediate readily amenable to functional group transpositions to give the desired oxamniquine analogues.

Manganese dioxide is known to be a mild and selective reagent for the oxidation of primary and secondary allylic and benzylic alcohols to the corresponding carbonyl compounds without significant further oxidation to carboxylic acids.[64] The reagent has become widely used in the synthesis of aromatic or heterocyclic aldehydes and ketones from substituted and unsubstituted benzyl alcohols.[65] El Hamouly[49] described the oxidation of oxamniquine to the aldehyde (**24**) in 89% yield using manganese dioxide. In our hands reaction of one equivalent of oxamniquine with ten equivalents of manganese dioxide in dichloromethane, overnight at room temperature, gave the aldehyde (**24**) in 73 % yield. (Scheme 2.3).



1

24 (73%)

Scheme 2.3

The IR spectrum showed an aryl carbonyl absorbance band at 1670 cm^{-1}. The proton NMR spectrum showed a carbonyl signal at δ10.13 ppm. Absence of the hydroxymethyl functionality, the good agreement of the melting point 99.3-99.8°C with the literature[49] value 99-100°C and elemental analysis consistent with the molecular formula $C_{14}H_{19}N_3O_3$ confirmed the oxidation of oxamniquine to the aldehyde.

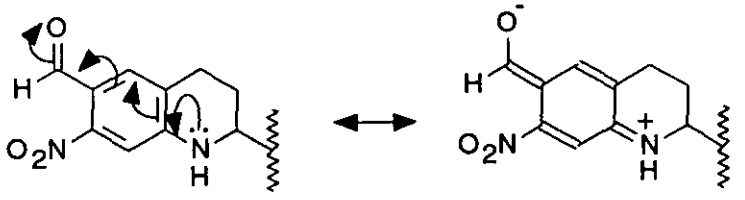
2.2.3. Preparation of protected derivatives of oxamniquine and oxamniquine aldehyde (24)

Oxamniquine aldehyde (**24**) is a multifunctional molecule containing in addition to the 6-aryl carbonyl, two basic nitrogen centres, the heterocyclic nitrogen (N_1) and the side chain nitrogen (N^2) respectively, and an nitro aromatic substituent *ortho* to the 6-C substituent. The basic NH groups are capable of donating or accepting protons and may interfere with a variety of reagents therefore necessitating the use of excess reagents to achieve the desired structural modifications at 6-C. It was therefore considered prudent to consider protection of the basic nitrogen centres in the aldehyde (**24**) before proceeding with the proposed structural modifications at position 6-C in order to isolate the reactivity of the carbonyl.

The criteria for the choice of the most appropriate protective group are the ease and selectivity of formation of the derivative, the stability of the derivative during the synthetic sequence, and the ease and selectivity of its removal. With the multiple functionalities present in the oxamniquine molecule, the stability of the protected derivative during the synthetic reactions and the selectivity of removal of the protective group to yield the modified oxamniquine analogue were of paramount importance to ensure minimal side reactions involving these functionalities. It was therefore desirable to identify an amine protective group which is easily and mildly introduced and removed at the end of the reaction sequence.

N-benzylation and N-acetylation are effective and widely used methods for the protection of amines in the synthesis of complex molecules.[66,67] However, the synthetic utility of both benzyl and acetyl protective groups is limited by the conditions used for their removal which may affect other labile groups in the molecule. The aromatic nitro group is especially labile to most of the methods used for the removal of the commonly used -N-benzyl protective groups:- catalytic hydrogenation using hydrogen and palladium on carbon, catalytic transfer hydrogenation using palladium on carbon and an organic molecule, methanoic acid, as the hydrogen source. On the other hand, the strong acid or basic conditions required for the removal of the acetyl group, to release the free base, are liable to affect other parts of the molecule.

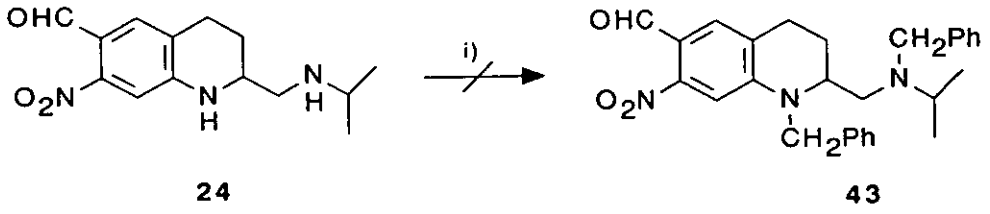
Notwithstanding the limitations associated with the use of these protective groups, it was considered necessary to examine the preparation of N-benzyl and N-acetyl derivatives of oxamniquine aldehyde in order to isolate the reactivity of the carbonyl. It was expected that this would facilitate selective modification at 6-C to give the desired oxamniquine analogues. It was particularly important to protect N_1 as delocalization of the nitrogen lone pair electrons into the aromatic system deactivates the *para*-conjugated carbonyl to nucleophilic attack as shown in Scheme 2.4. It was anticipated that formation of the benzylamine or the acetamide at N_1 would increase the reactivity of the carbonyl group as the nitrogen lone pair electrons are less available for delocalization.



Scheme 2.4

(a) Attempted synthesis of 6-methanoyl-1-N-benzyl-2-(N-benzyl-N-isopropyl-aminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline, N_1,N^2-dibenzyl oxamniquine aldehyde (43)

We attempted to prepare the N,N-dibenzyl derivative (**43**) of the aldehyde (**24**) using three methods as shown in Scheme 2.5.



24 **43**

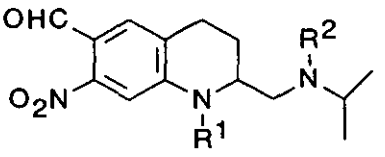
i) $PhCH_2Cl$, K_2CO_3, aq. EtOH, reflux, 30h,
or $PhCH_2Cl$, NaH, DMF, RT, 24h,
or $PhCH_2Br$, DMF, RT, 24h.

Scheme 2.5

(i) Reaction with benzyl chloride using potassium carbonate as base

The reaction of an aqueous ethanolic solution of the aldehyde (**24**) with three mole equivalents of potassium carbonate at room temperature followed by treatment, at reflux, with a three molar ethanolic solution of benzyl chloride and subsequent refluxing for 30 hours according to the method described by Velluz[68] yielded a brown oil. Column chromatography on silica gel using chloroform:hexane:ethanol (10:10:10) as the eluent yielded three components: the unreacted aldehyde (**24**), benzyl chloride and a brown oil. Preliminary characterization of the oil by TLC showed it to be a single component the IR spectrum of which showed NH absorbance at 3372 cm^{-1}. The 60MHz, $CDCl_3$ proton NMR spectrum showed the following important chemical shifts: a one-proton singlet at δ10.15 ppm which was assigned as the aryl aldehyde proton; two one-proton singlets at δ7.55 and 6.7 ppm were assigned as the aromatic signals at 5-C and 8-C respectively; a third singlet at δ7.3 ppm which integrated to five protons was assigned to the aromatic protons of the benzyl substituent. A two-proton doublet, with coupling constant 5Hz, at δ3.5-3.65 ppm was assigned as the N-benzyl methylene protons. In addition two deuterium oxide-exchangeable one-proton singlets at δ5.45 and 1.2 ppm were assigned as 1-N and N^2 protons respectively.

Although the NMR spectrum showed the presence of an N-benzyl substituent, the presence of signals characteristic of the nitrogen protons at δ5.45 and 1.2 ppm as well as the NH absorbance at 3372 cm^{-1} in the IR spectrum suggested that benzylation of the aldehyde resulted in the formation of two mono-benzylated products (**44**) and (**45**), which chromatograph together.



R^1	R^2	
CH_2Ph	H	(44)
H	CH_2Ph	(45)

(ii) Reaction with benzyl chloride using sodium hydride as the base

The reaction of the aldehyde (**24**) with benzyl chloride using of sodium hydride as the base in an aprotic solvent, according to the method described by Srinivasan,[69] was investigated. The aldehyde dissolved in dry DMF was deprotonated by treatment with three mole equivalents of sodium hydride at 100°C for two hours. Subsequent cooling to room temperature, followed by addition of three moles of dry benzyl chloride in DMF and overnight stirring at room temperature under nitrogen yielded a brown oil after aqueous work up. TLC of the reaction product showed the presence of several components including the unreacted aldehyde (**24**). Further separation and identification of the reaction product was attempted.

(iii) Reaction with benzyl bromide

The reaction of the aldehyde (**24**) with benzyl bromide in DMF at room temperature according to the method of Chapman[70] was also investigated. Dropwise addition of two mole equivalents of benzyl bromide dissolved in dry DMF to a DMF solution of the aldehyde (**24**), under nitrogen, followed by overnight stirring at room temperature and basic extraction yielded an orange-coloured oil. TLC of the crude reaction product showed a complex mixture including the aldehyde (**24**). Further separation and identification of the components was not attempted.

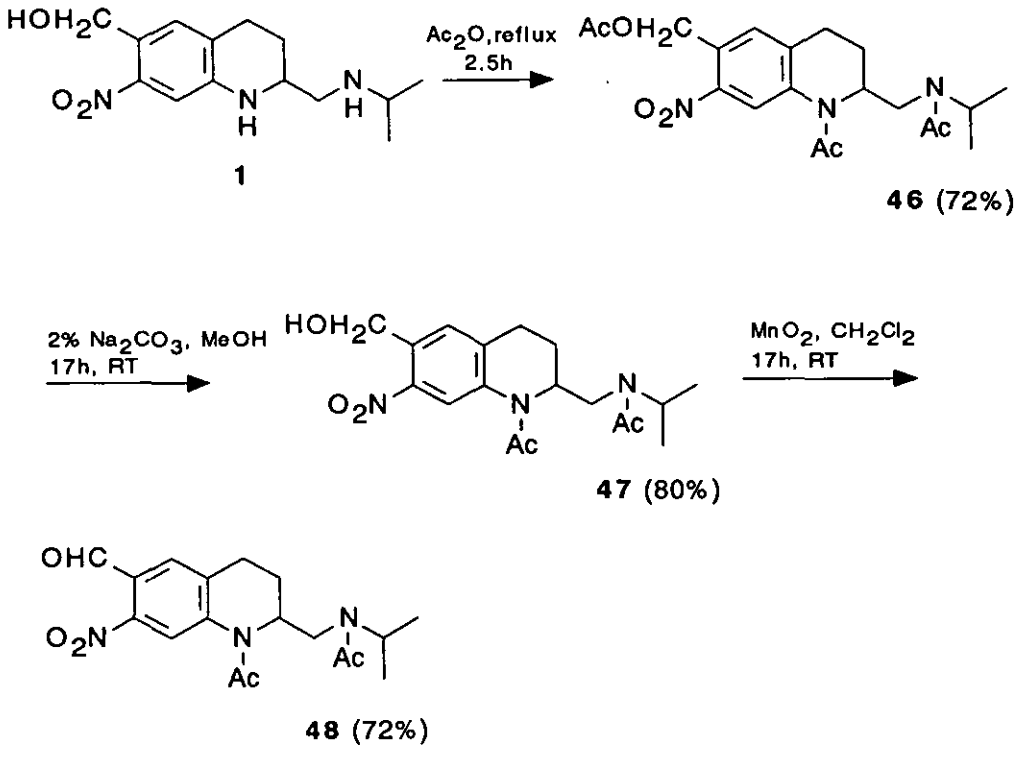
The attempts to prepare the N,N-dibenzylated derivative (**43**) using the three methods were unsuccessful. In all cases a mixture of products was obtained and the unreacted aldehyde (**24**) was recovered. Although evidence for the formation of two monobenzylated derivatives (**44**) and (**45**) in one of the reactions was obtained, no spectral properties characteristic of the desired dibenzylated derivative (**43**) were evident even in the crude reaction products.

The bulky isopropyl substituent and the tetrahydroquinoline residue probably hinder benzylation at the side chain nitrogen (N^2). A factor for the ring nitrogen (N_1) could be the influence of the *para*-conjugated aldehyde (Scheme 2.4). The lone pair electrons of N_1 are delocalized into the aromatic system decreasing the

nucleophilicity of N_1. Thus perhaps quantitative benzylation at both positions resulting in the formation of the dibenzyl derivative (**43**) may not be achieved.

(b) N_1,N^2 diacetyl derivatives of oxamniquine

The N_1,N^2 diacetyl derivatives of oxamniquine were prepared as shown in Scheme 2.6.



Scheme 2.6

(i) 6-Acetoxymethyl-1-N-acetyl-2-(N-acetyl-N-isopropylaminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline (**46**)

The novel peracetylated derivative (**46**) of oxamniquine was readily prepared in 72 % yield by 2.5 hours refluxing of oxamniquine with excess acetic anhydride according to the method described by Baxter.[71] The peracetylated derivative (**46**) was recrystallized, from petroleum ether : ethanol, as yellow crystals, melting point 134.0-134.6°C. The chloroform IR spectrum showed the presence of three strong absorbances at 1740, 1660 and 1634 cm^{-1} which were assigned as the acetate carbonyl, the amide carbonyl and amide N-H bending absorbances respectively. The presence of three acetyl functionalities was confirmed by the NMR spectrum which showed three, three-proton singlets at δ2.22, 2.17 and 2.15 ppm which were assigned as the acetyl protons of the amide at N_1, the acetate at 9-C and the amide at N^2 respectively. Chemical ionization mass spectrometry

gave the measured mass 406.1980 for the (M+H) peak from the molecular formula $C_{20}H_{27}N_3O_6$ which was confirmed by elemental analysis.

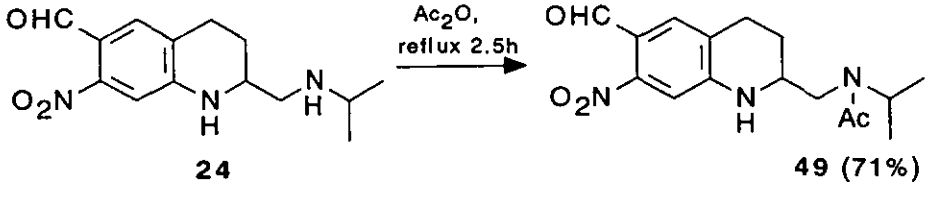
(ii) 6-Hydroxymethyl-1-N-acetyl-2-(N-acetyl-N-isopropylaminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline (**47**)

Hydrolysis of the peracetylated derivative (**46**) with 2% sodium carbonate in methanol, overnight, at room temperature yielded the novel N_1,N^2 diacetyl derivative (**47**) of oxamniquine in 80 % yield. The diacetyl derivative (**47**) was recrystallized from ethanol as yellow crystals, melting point 165.6-166.1°C. The hydrolysis of the acetate functionality was confirmed by the disappearance of the acetate carbonyl absorbance at 1740 cm^{-1} in the IR spectrum and the appearance of only two acetyl singlets at δ2.15 and 2.21 ppm in the proton NMR spectrum. The presence of the amide functionality was confirmed by the carbonyl absorbance at 1654 cm^{-1} and amide N-H stretching at 1634 cm^{-1}. Chemical ionization mass spectrometry gave the measured mass 364.1870 for the (M+H) peak and elemental analysis was consistent with the molecular formula $C_{18}H_{25}N_3O_5$.

(iii) 6-Methanoyl-1-N-acetyl-2-(N-acetyl-N-isopropylaminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline (**48**)

Oxidation of the diacetyl derivative (**47**) with excess manganese dioxide in dichloromethane, overnight at room temperature, according to the method of El Hamouly[49] yielded the N_1,N^2 diacetyl derivative (**48**) of oxamniquine aldehyde in 72 % yield. The aldehyde (**48**) was recrystallized as a yellow solid from dichloromethane hexane, melting point 137-138.6°C. The IR spectrum showed amide absorbance bands at 1686 and 1628 cm^{-1} and a carbonyl absorbance band at 1664 cm^{-1}. The proton NMR spectrum showed two three-proton singlets at δ2.16 and δ2.27 ppm representing the acetyl groups at N^2 and N_1 respectively. A one-proton singlet at δ10.38 ppm was assigned as the aryl conjugated aldehyde obtained on oxidation of the benzylic alcohol at 6-C.

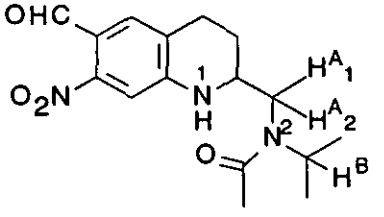
(c) 6-Methanoyl-2-(N-acetyl-N-isopropylaminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline, N^2-acetyl- oxamniquine-6-aldehyde (49)



Scheme 2.7

The novel N^2-acetyl oxamniquine aldehyde (**49**) was prepared in 71 % yield by refluxing the aldehyde (**24**) with excess acetic anhydride for 2.5 hours according to the method of Baxter.[71] (Scheme 2.7) The IR spectrum revealed absorption peaks consistent with a monoacetylated derivative of oxamniquine aldehyde, the peaks at 3268, 1658 and 1632 cm^{-1} being representative of the secondary amine, aryl aldehyde and amide carbonyl functionalities respectively.

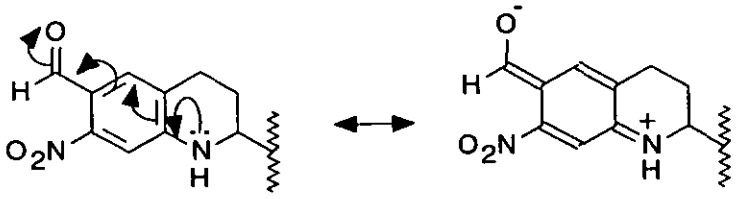
The proton NMR showed a three proton singlet at δ2.22 ppm corresponding to the methyl protons of the acetyl group. The multiplet of the side chain proton B was observed to be shifted downfield from δ2.79-2.91 ppm in the aldehyde (**24**) to δ4.06-4.11 ppm in the acetyl derivative (**49**). The chemical shifts of the protons A_1 and A_2 were also shifted downfield from δ2.97-3.03 ppm and δ2.42-2.51 ppm respectively in the aldehyde (**24**) to δ3.61-3.73 ppm and δ3.05-3.11 ppm respectively in the acetyl derivative (**49**). A downfield shift in the chemical shift of the 1-NH in the acetyl derivative (**49**), from δ6.00 ppm in the aldehyde (**24**) to δ6.98 ppm, where it overlaps the aromatic 8-H, was also noted. These downfield shifts suggests that in the acetyl derivative (**49**) the protons: B, A_1, A_2 and 1-NH, are subject to strong deshielding effects arising from the introduction of an acetyl group at N^2. The downfield shift of 1-NH suggests possible hydrogen bonding between this proton and the oxygen of the acetyl functionality at the side chain nitrogen, N^2.



A_1 δ3.61-3.73 ppm
A_2 δ3.05-3.11 ppm
B δ4.06-4.11ppm
1-H δ6.98 ppm

49

As previously noted it was expected that N^2 would be more basic than N_1 due to the influence of the 6-carbonyl which is *para*-conjugated with N_1 (Scheme 2.4). The lone pair electrons of N_1 are therefore delocalized into the aromatic system decreasing the nucleophilicity of N_1. Acetylation therefore occurs exclusively at the secondary amine, N^2.

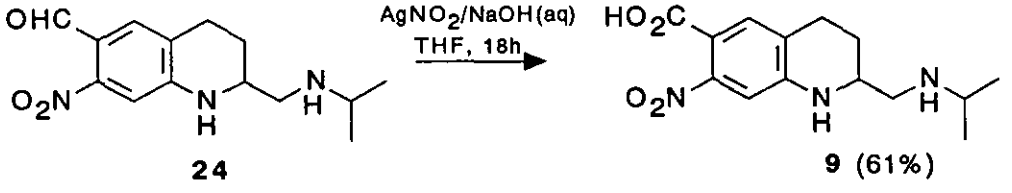


Scheme 2.4

No further attempts to prepare other N-protected derivatives of oxamniquine aldehyde (**24**) were pursued and the diacetyl aldehyde derivative (**48**) was used later (Section 2.2.8).

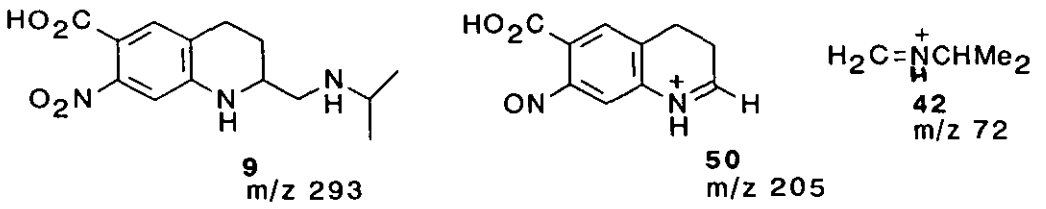
2.2.4. 6-Carboxy-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydro-quinoline, Oxamniquine 6-carboxylic acid (9)

The acid metabolite of oxamniquine (**9**) has previously been isolated from the urine of rats dosed with oxamniquine (**1**)[21] or UK 3883 (**11**).[22] We however, decided to synthesize this metabolite by oxidation of oxamniquine aldehyde (**24**). Silver oxide oxidation is a mild and selective method for the oxidation of aldehydes to carboxylic acids.[72,73] The oxidation of the aldehyde (**24**) using silver oxide was therefore undertaken as shown in Scheme 2.8.



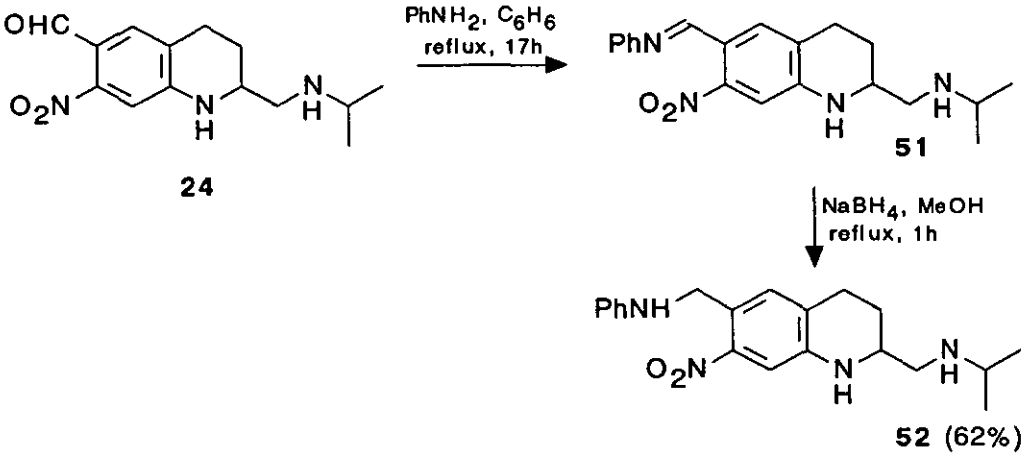
Scheme 2.8

Reaction of one equivalent of the aldehyde (**24**) with one equivalent of silver oxide, generated *in situ* from sodium hydroxide and silver nitrate, at room temperature for eighteen hours, gave the acid (**9**) which was isolated as the hydrochloride salt in 61 % yield. The IR spectrum showed strong carbonyl absorbance at 1692 cm^{-1} due to the carboxylic acid. The deuterium oxide proton NMR spectrum showed the isopropyl substituent as two doublets at δ1.16-1.19 ppm, the aromatic protons: 8-H at δ6.87 ppm and 5-H at δ7.32 ppm. Electron impact mass spectroscopy gave the molecular ion 293. The following fragments were also detected m/z 205 (15%) and 72 (100%) which may be assigned as the fragments (**50**) and (**42**) respectively.



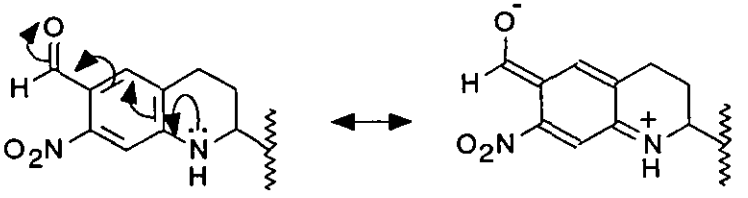
2.2.5. 6-(1-Phenylaminomethyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine arylamine (52)

The anil (**51**) was obtained from the aldehyde (**24**) by condensation with aniline. (Scheme 2.9).



Scheme 2.9

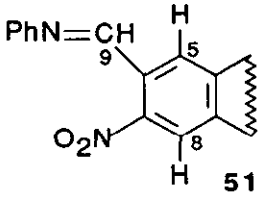
Although *m* -nitro substituted anils are readily prepared by condensation of the nitro aldehyde with aniline in aqueous ethanol,[74] initial attempts to prepare the anil by this method resulted only in partial conversion of the aldehyde (**24**) to the anil (**51**). The anil (**51**) was successfully prepared by, overnight reaction, of one equivalent of the aldehyde (**24**) with four equivalents of aniline in refluxing benzene with azeotropic removal of water. The forcing conditions were required for the reaction because of the influence of the *para* conjugated N_1. It is presumed that this decreases the susceptibilty of the aldehyde to nucleophilic attack. (Scheme 2.4)



Scheme 2.4

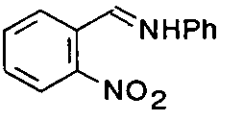
The IR spectrum of the crude anil showed NH absorbance at 3380 cm^{-1} and aromatic C-H bending vibrations at 752 and 694 cm^{-1} characteristic of a monosubstituted benzene ring. A strong absorbance band at 1668 cm^{-1} was thought to be due to C=N stretching vibrations. The 60MHz $CDCl_3$ proton NMR spectrum of the crude anil (**51**) showed the following important chemical shifts: a one-proton singlet at δ8.80 ppm which was assigned as the imine proton at 9-C and two multiplets, integrating to a total of five protons, at δ7.1-7.4 ppm and δ6.42-6.87 ppm which were assigned as the aromatic protons of the aniline nucleus. The aromatic proton, 5-H, was noted to be shifted downfield to δ7.90 ppm due to the deshielding effect of the imine substituent at 6-C.

Attempts to isolate and purify the anil by chromatography and recrystallization were, however, unsuccessful. The anil (**51**) was unstable and readily decomposed to the aldehyde (**24**) and aniline in the presence of water. *o* -Nitrobenzaniline (**53**) has been reported to be very photosensitive.[75] It is possible that the same may be true of the oxamniquine anil (**51**).



51

5-H δ7.90 ppm
8-H δ7.01 ppm
9-H δ8.80 ppm

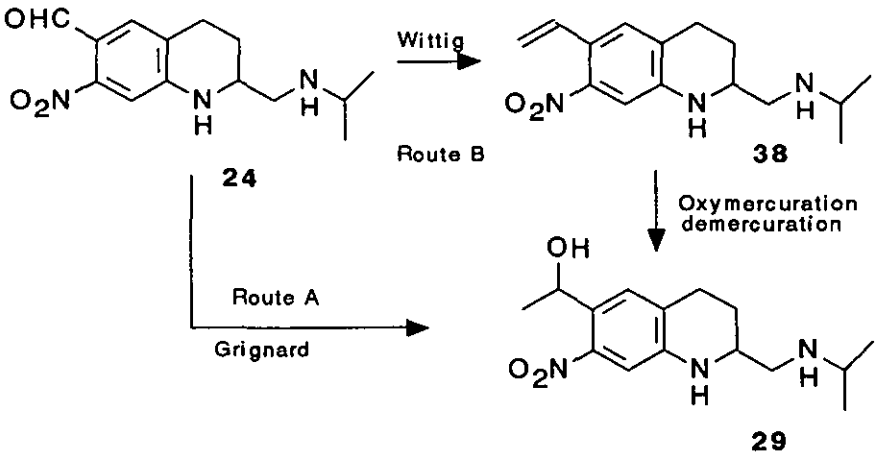


53

The crude anil was therefore reduced to the arylamine (**52**) without further purification. Sodium borohydride reduction of the anil (**51**) in refluxing methanol, as shown in Scheme 2.9 gave the arylamine (**52**) in good yield. The amine (**52**) was isolated as an oil which solidified on cooling to a low melting red solid. Recrystallization of the amine was unsuccessful, all attempts resulted in the isolation of decomposition products. However, spectral analysis of the solid gave spectral characteristics consistent with the arylamine (**52**). The proton NMR spectrum showed the disappearance of the aldehyde functionality at δ10.13 ppm and the appearance a two-proton singlet at δ4.48 ppm which was assigned to the benzylic methylene protons at 9-C. Two deuterium oxide-exchangeable, one-proton singlets, at δ4.30 and δ5.30 ppm were assigned to the two protons from the benzylic amines. In addition three new signals:- a two-proton doublet at δ6.60 ppm, coupling constant 8.1Hz, a one-proton triplet at δ6.67-6.73 ppm, coupling constants 8.1 and 6.1 Hz, and a two-proton triplet at δ7.12-7.18 ppm, coupling constants 8.1 and 6.1 Hz, were observed and were assigned to the five aromatic protons of aniline. Electron impact mass spectrometry gave the measured molecular mass 354.2033 required for $C_{20}H_{26}N_4O_2$.

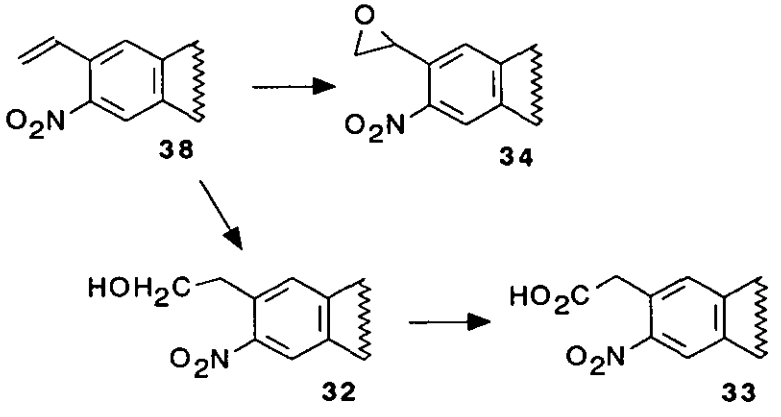
2.2.6. 6-(1-Methyl-1-hydroxymethyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine secondary alcohol homologue (29)

Scheme 2.10 shows the strategy proposed for the preparation of the secondary alcohol homologue of oxamniquine (29)



Scheme 2.10. Proposed routes to the synthesis of oxamniquine secondary alcohol (29)

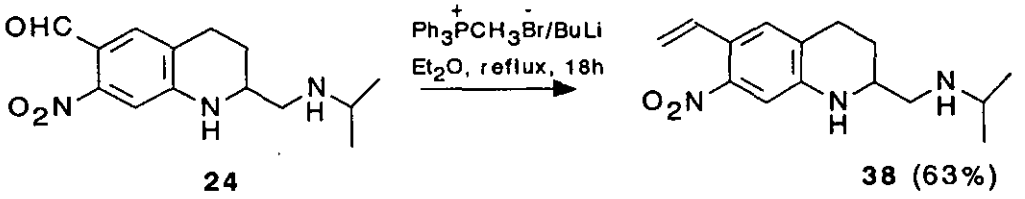
It was anticipated that the alcohol (29) could be obtained from the aldehyde (24) either by Route A, a low temperature Grignard reaction,[76] or via Route B, an oxymercuration demercuration reaction of the olefin (38)[77] available from the aldehyde (24) by the classical Wittig reaction.[78] However, since nitro groups and amines are known to react readily with Grignard reagents[76] Route B via the olefin (38) was initially considered more favourable. In addition it was expected that the olefin (38) would provide a versatile synthetic intermediate giving rise to the epoxide (34), the one carbon homologue of oxamniquine the alcohol (32) and the acid derivative (33) which is the one carbon homologue of the acid metabolite (9) of oxamniquine (Scheme 2.11).



Scheme 2.11

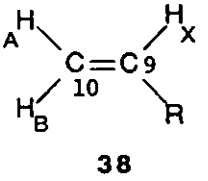
(a) 6-(1-Ethenyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetra-hydroquinoline, Oxamniquine olefin (38)

The Wittig reaction is a versatile method for 1-C homologation of carbonyl carbon compounds via the formation of alkenes. We used the classical Wittig reaction[78] as a means of homologation oxamniquine at position 6 as shown in Scheme 2.12.

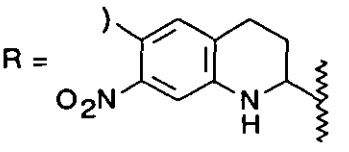


Scheme 2.12

Reaction of one equivalent of oxamniquine aldehyde (**24**) with four equivalents of methyltriphenylphosphonium bromide in dry diethyl ether using n-butyl lithium as base, gave the olefin (**38**) in 63 % yield. The olefin was isolated as a dark red oil which solidified to a low melting solid. Attempts to recrystallize the olefin were, however, unsuccessful yielding only polymeric and decomposition materials. Spectroscopic analysis of this olefin gave results consistent with the desired compound (**38**). The proton NMR spectrum showed the appearance of a 12 line signal, inte.grating to three protons, at δ7.00-7.12, 5.49-5.57 and 5.19-5.24 ppm, which is characteristic of an ABX olefinic signal. The double doublet at δ7.00-7.12 ppm with coupling constants 11 and 17.5 Hz was assigned as the olefinic proton at 9-C, the X proton of the ABX system. The double doublet at δ5.49-5.57 ppm, coupling constants 17.5 and 1 Hz, was assigned as the *trans* olefinic proton at 10-C, the B proton of the ABX signal. The double doublet at δ5.19-5.24 ppm, coupling constants 11 and 1 Hz was assigned as the A proton of the ABX signal, the *cis* olefinic proton at 10-C.



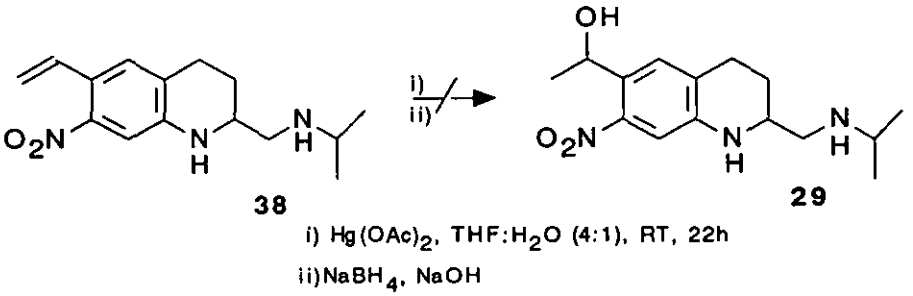
38



H_A δ5.19-5.24 ppm, dd J11 and 1 Hz
H_B δ5.49-5.57 ppm, dd J17.5 and 1 Hz
H_X δ7.00-7.12 ppm, dd J11 and 17.5 Hz

(b) Oxymercuration-demercuration reaction of 6-(1-ethenyl)-2-N-isopropyl-aminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline (38)

The oxymercuration of olefins in aqueous THF followed by *in situ* reduction of the organomercurial by alkaline sodium borohydride provides a convenient procedure for the Markovnikov hydration of carbon carbon double bonds.[79] We investigated the oxymercuration-demercuration reaction of the olefin (**38**) as a possible route to the synthesis of the secondary alcohol homologue of oxamniquine (**29**). (Scheme 2.13).

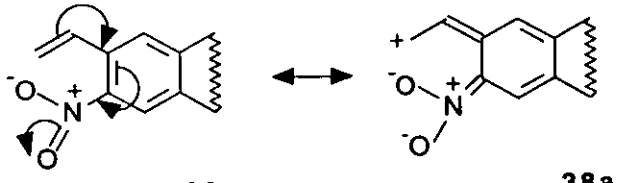


Scheme 2.13

Reaction of equimolar ratios of the olefin (**38**) and mercuric acetate in aqueous tetrahydrofuran followed by *in situ* demercuration by reduction with alkaline sodium borohydride did not yield the desired alcohol (**29**). The olefin was recovered unchanged even when the more hydrophobic solvent composition of THF : water (4:1) was used in place of THF : water (3:1) and the time for the oxymercuration reaction was increased from 22 hours to 66.5 hours. The yellow precipitate, mercuric oxide, obtained on addition of THF to the aqueous mercuric acetate solution did not dissolve on addition of the olefin (**38**) even after prolonged stirring at room temperature. The time for disappearance of this yellow precipitate has been correlated with extent of the reaction. The change in colour provides an approximate measure of the reaction rate. Brown[79] noted that in general the reaction had gone to 60 % completion when all of the yellow precipitate or suspension had vanished. A darkening of the suspension with deposition of a metallic grey solid was however, observed in the reaction.

The addition of mercury salts to carbon carbon double bonds is electrophilic and the presence of electron-acceptor substituents hinders the reaction.[80] Steric factors as well as the conjugation of the double with a benzene ring are known to be critical in determining the reactivity of olefins to oxymercuration.[81] In the olefin (**38**) the double bond is conjugated to an aromatic ring. This decreases the nucleophilicity of the double bond and also has a steric effect. The double bond is

also conjugated to an *ortho* nitro group which may serve to decrease the susceptibility of the olefin to electrophilic attack as shown in Scheme 2.14. This nitro group also sterically hinders attack on the double bond making the olefin (**38**) particularly unreactive.



38 **38a**

Scheme 2.14

The hydrophobic nature of the olefin (**38**) may also contribute to its lack of reactivity. High molecular weight olefins have previously been converted to the corresponding Markovnikov alcohols using less aqueous solvent compositions such as THF : water ratios (3:1) and (4:1).[79] The use of more aqueous solvents with hydrophobic olefins led to slower reaction times presumably due to the limited solubility of the olefin in the more aqueous system. However, in our case even the (4:1) THF : water mixture was ineffective in solubilising the olefin (**38**).

The appearance of the metallic grey deposit during the oxymercuration stage of the reaction suggested the reduction of the mercuric acetate to metallic mercury had occurred. It is possible that the basic nitrogens in the olefin react preferentially, reducing mercuric acetate to mercury.

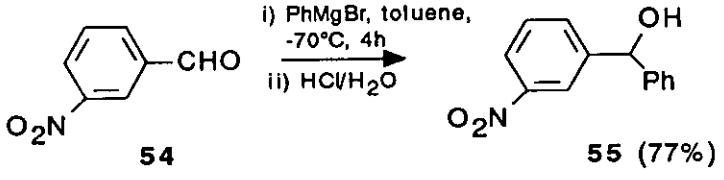
Thus the presence of the *ortho* nitro substituent, the aromatic ring as well as the basic nitrogen centres in the olefin (**38**) may account for the observed unreactivity of this compound.

Although the oxymercuration reaction is acid catalysed and the use of 1.5 ml of 70 % perchloric acid per mole of mercuric acetate has been advocated to accelerate the reaction,[82] the use of acid catalysis was not considered prudent as protonation would increase the electron withdrawing properties of N_1 and therefore decrease the reactivity of the olefin.

Since attempts to obtain the alcohol homologue (**29**) via oxymercuration and demercuration of the olefin (**38**) were unsuccessful, attention was focused on Route A, the Grignard reaction.

(c) Reaction of oxamniquine aldehyde (24) with methyl titanium trichloride

Grignard reagents are indispensable tools in preparative organic chemistry when it is necessary to transfer an alkyl group to an electrophilic centre.[83] The Grignard reaction therefore provides an alternative route for the synthesis of homologous alcohols from aldehydes. However, Grignard reagents are considered to be insufficiently selective, owing to their high reactivity and basicity.[83] The reagents are sometimes known not to be able to distinguish among different carbonyl functions (aldehyde, ketone, amide, ester) nor to give selective attack to the carbonyl group in the presence of nitro, cyano and iodo functions. Low temperature Grignard reactions have however, been used in the synthesis of carbinols from nitro-substituted carbonyl compounds.[84] The reaction, at -70°C, of phenylmagnesium bromide with *m* -nitrobenzaldehyde (**54**) gave phenyl *m* -nitrophenyl carbinol (**55**) in 77 % yield. (Scheme 2.15). However, with butylmagnesium bromide only tarry products were obtained.

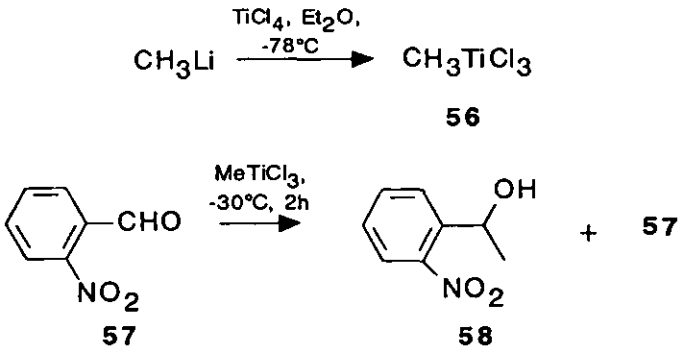


54 **55** (77%)

Scheme 2.15

Preparation of the alcohol (**29**) by a low temperature Grignard reaction of the aldehyde (**24**) with methyl magnesium bromide was not attempted. Rather, methyl titanium trichloride (**56**) was considered a more promising reagent for the preparation of the desired alcohol. Methyl titanium trichloride, a non basic, highly selective Grignard analogue, adds chemoselectively and stereoselectively to aldehydes and ketones even in the presence nitro, cyano or ester groups which do not interfere with carbonyl addition.[85] The reagent (**56**) discriminates between aldehyde and ketones resulting in complete aldehyde selectivity.

In a preliminary experiment, we examined the reaction of *o*-nitrobenzaldehyde (**57**) with three equivalents of methyl titanium trichloride at -30°C for two hours. (Scheme 2.16)

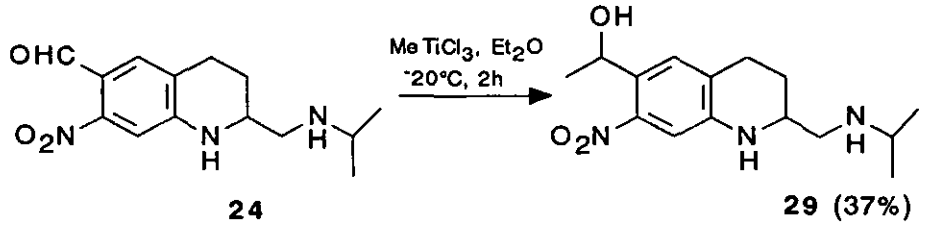


Scheme 2.16

The reagent (**56**) was prepared *in situ* by the addition, under nitrogen, of an ethereal solution of methyl lithium (one part) to a dichloromethane solution of titanium tetrachloride (one part) at -78°C. The solution was then allowed to warm up to -30°C.

Inverse addition of an ethereal solution of *o*-nitrobenzaldehyde to three mole equivalent of the reagent (**56**) at -30° C followed by reaction for two hours, under nitrogen, and subsequent aqueous work up gave a mixture of the aldehyde (**57**) and the corresponding carbinol (**58**). The IR spectrum showed the presence of a broad O-H absorbance band at 3420 cm^{-1} and strong carbonyl absorbance at 1700 cm^{-1}. The 60 MHz proton NMR spectrum of the crude product showed a one-proton quartet at δ5.20-5.54 ppm characteristic of a benzylic methine proton and a methyl doublet at δ3.2 ppm indicating the presence of the carbinol (**58**). A singlet at δ10.38 ppm characteristic of an aryl aldehyde proton confirmed the presence of both the aldehyde (**57**) and the carbinol (**58**). The approximate relative proportions of the unreacted nitrobenzaldehyde (**57**) and carbinol (**58**) in the crude product, as calculated from the NMR spectrum, were 40 and 60 % respectively.

We investigated the reaction of the aldehyde (**24**) with methyl titanium trichloride as shown in Scheme 2.17.



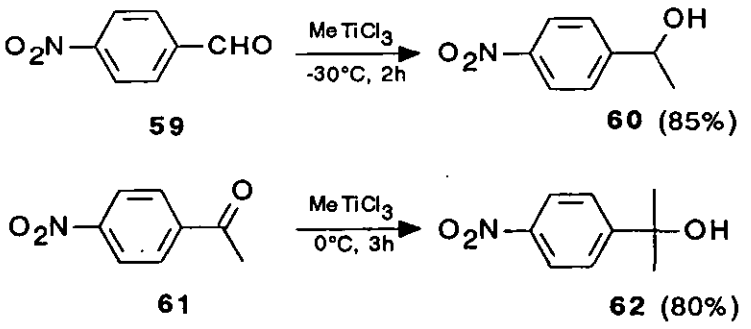
Scheme 2.17

Initial attempts using inverse addition of five molar equivalents of the reagent (**56**) to an ethereal solution of the aldehyde (**24**) at -30°C followed by stirring at

-30°C for two hours and aqueous work up gave the desired alcohol (**29**) in 24 % yield and the unreacted aldehyde (**24**).

In attempt to increase the yield of the alcohol (**29**) the reaction temperature and the order of addition of the reactants were changed. Addition of one mole equivalent of an ether solution of the aldehyde (**24**) to five mole equivalents of an ethereal solution of the methyl titanium trichloride reagent at -20°C and subsequent reaction at -20° for two hours followed by aqueous work up increased the yield of the alcohol (**29**) to 37 %.

Attempts to increase either the temperature higher than -20°C or the reaction time to four hours resulted in the formation of side products with no significant increase in the yield of the alcohol (**29**) and poor recovery of the unreacted aldehyde (**24**). Reetz[85] reacted *p*-nitrobenzaldehyde (**59**) with molar equivalents of titanium trichloride for two hours at -30°C to give the carbinol derivative (**60**) in 85% yield. Using a higher temperature, 0°C, and a longer reaction time, three hours, reaction of the less reactive ketone (**61**) with methyl titanium trichloride gave the tertiary alcohol (**62**) in 80 % yield. (Scheme 2.18)

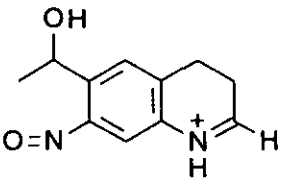


Scheme 2.18

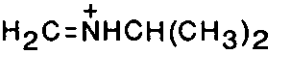
We however, we were not able to increase the yield of the alcohol (**29**). The poor yields obtained with oxamniquine aldehyde (**24**) may be due to the steric hindrance offered by the *ortho* nitro group to attack of the adjacent carbonyl by the titanium reagent (**56**) as well as the influence of the basic nitrogens.

The alcohol (**29**) was recrystallized as a yellow powder, melting point 152.0-153.5°C. Spectral analysis gave results consistent with spectral characteristics expected for the alcohol (**29**). The proton NMR spectrum showed the presence of a three-proton doublet centred at δ1.53 ppm, coupling constant 6.4 Hz, which was assigned to the 10-C methyl protons. A one-proton quartet at δ5.20-5.28 ppm,coupling constant 6.4 Hz, was assigned as the proton at the new chiral centre

9-C. In some samples the 5-H proton appeared as two singlets at δ7.02-7.03 ppm signifying the presence of a pair of diastereoisomers that arise as a result of the new chiral centre at 9-C. Electron impact mass spectrometry confirmed the presence of the molecular ion 293 with the major fragments m/z 205 and m/z 72 which may be assigned as the ion fragments (**63**) and (**42**) respectively. The measured molecular mass 293.1730 was consistent with the molecular formula $C_{15}H_{21}N_3O_3$.



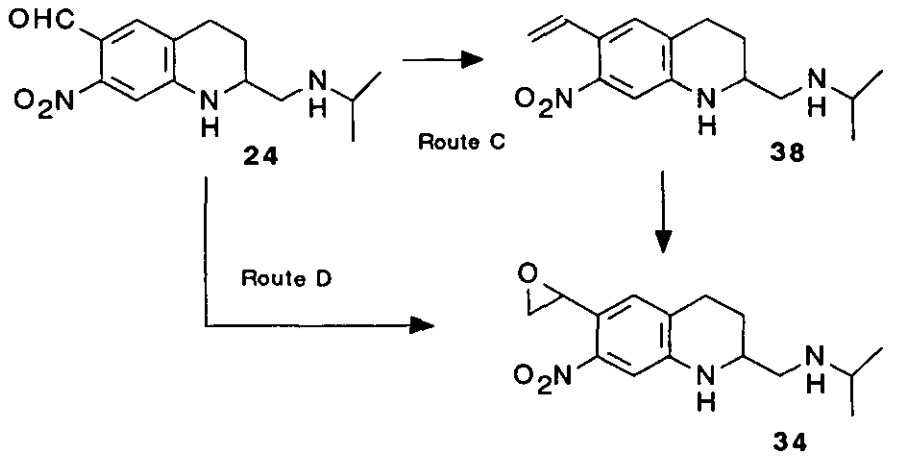
63



42

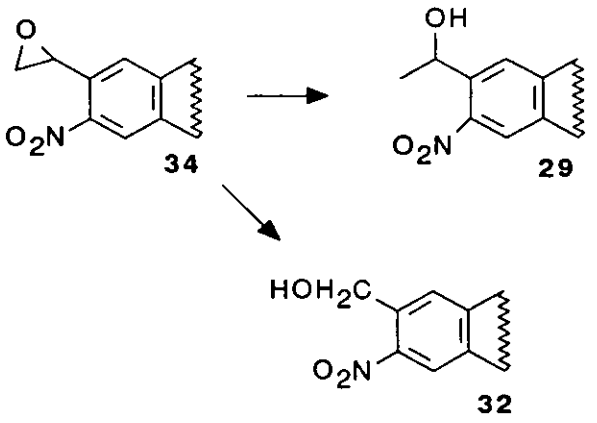
2.2.7. 6-(Oxiran-2-yl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydro-quinoline, Oxamniquine epoxide (34)

As previously noted it was initially expected that the oxamniquine epoxide (**34**) could be obtained by epoxidation of the olefin (**38**), Route C Scheme 2.19. Alternatively the epoxide (**34**) could be available by the reaction of the aldehyde (**24**) with a methylene transfer reagent as shown by Route D, Scheme 2.19.



Scheme 2.19 Proposed routes to the synthesis of oxamniquine epoxide

It was envisaged that the epoxide (**34**) would be a potent alkylating agent as well as a reactive synthetic intermediate readily converted to the secondary alcohol homologue (**29**) of oxamniquine and the homologous alcohol (**32**), Scheme 2.20.

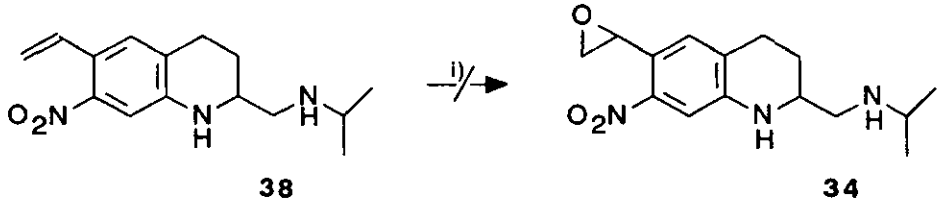


Scheme 2.20

(a) Attempted epoxidation of oxamniquine olefin (38)

(i) Alkaline hydrogen peroxide oxidation

The direct conversion of alkenes into epoxides can be achieved by various methods including oxidation with organic peroxy acids and oxidation with hydrogen peroxide in the presence of metal catalysts.[86] However, for electrophilic alkenes, where the olefinic bond is conjugated with an electron withdrawing group which decreases the reactivity with peroxy acids, epoxide formation is readily achieved by alkaline hydrogen peroxide or alkaline tertiary butylhydroperoxide.[86] We investigated the epoxidation of oxamniquine olefin (**38**) by alkaline hydrogen peroxide which has previously been used for the epoxidation of 2-methyl-2-nitro styrene to give the nitrostyrene epoxide in 67 % yield.[87] Alkaline hydrogen peroxide oxidation of the olefin (**38**) in methanol, at 15°C for one hour, using 1.5 molar equivalents of hydrogen peroxide gave the unchanged olefin (**38**). (Scheme 2.21)



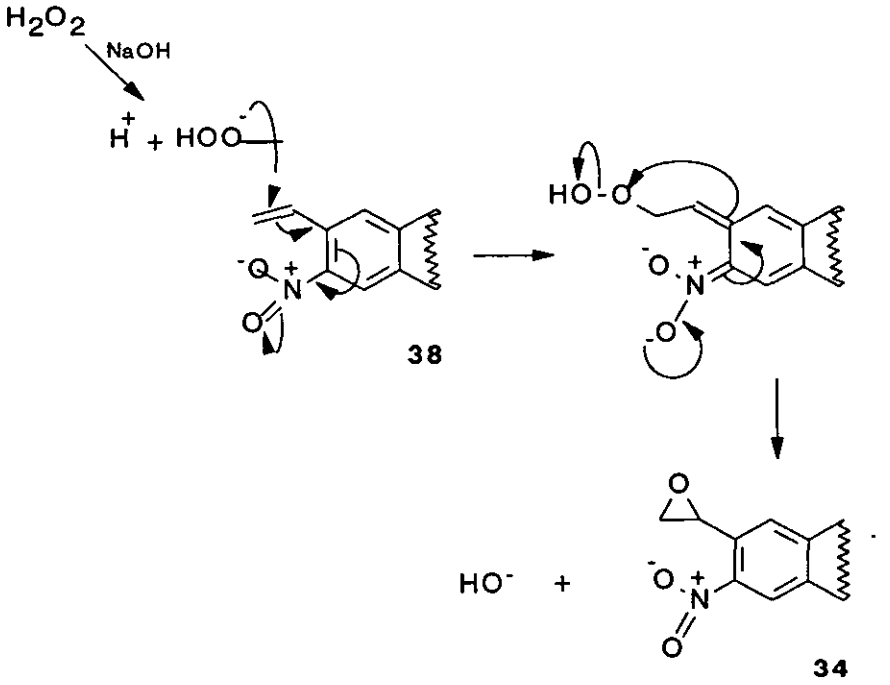
38 **34**

i) either H_2O_2, NaOH, MeOH, 15°C, 1h
or UHP, Ac_2O, CH_2Cl_2, RT, 30h

Scheme 2.21

The olefin (**38**) was recovered unchanged even after reacting with 8.8 equivalents of alkaline hydrogen peroxide for 120 hours at 15°C.

Although, alkaline hydrogen peroxide has been used for the epoxidation of deactivated styrenes,[87] the epoxidation of oxamniquine olefin (**38**) by the accepted mechanism,[86] Scheme 2.22, would proceed via destruction of aromaticity. This would require vigorous reaction conditions. This highly unfavourable prerequisite may account for the failure to generate the epoxide (**34**) from the olefin (**38**).

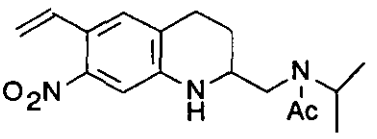


Scheme 2.22

(ii) Oxidation with urea-hydrogen peroxide

Urea-hydrogen peroxide (UHP) alone or in combination with carboxylic anhydrides has been shown to be a valuable alternative to anhydrous hydrogen peroxide in the epoxidation of alkenes.[88] We investigated the oxidation of the olefin (**38**) with UHP in combination with acetic anhydride. (Scheme 2.21)

The reaction of one equivalent of the olefin (**38**), dissolved in dichloromethane, with 8.6 equivalents of UHP in combination with 2.2 equivalents of acetic anhydride and in the presence of sodium phosphate buffer at room temperature for 30 hours was nonselective. A mixture of products was obtained. Analysis of the IR and proton NMR spectra of the reaction mix showed no evidence of an epoxide residue. The reaction product did however, show the presence of an N-acetyl compound which could be the acetamide (**64**). Partial acetylation of the olefin (**38**) by acetic anhydride to yield the acetamide (**64**) is not surprising. Had evidence of an epoxide residue been obtained it would have been necessary to repeat the reaction using an N-acetyl derivative of the olefin (**38**). The failure to epoxidize the olefin (**38**) was surprising.

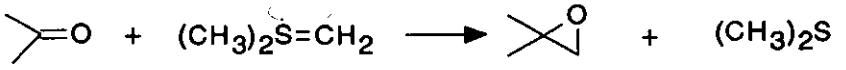


64

(b) Reaction of oxamniquine aldehyde (24) with dimethylsulfonium methylide

The oxidation of *o* -nitrobenzaldehyde (**57**) to *o*-nitrostyrene oxide (**66**) was considered as a model reaction for the preparation of oxamniquine epoxide (**34**) from the aldehyde (**24**). Literature methods reported for the synthesis of *o*-nitrostyrene oxide (**66**) include: (i) the reaction of *o*-nitrobenzaldehyde with diazomethane[89] and (ii) α-bromination of *o*-nitroacetophenone followed by reduction of the resulting bromoketone to the corresponding bromohydrin and cyclization to the epoxide.[90,91]

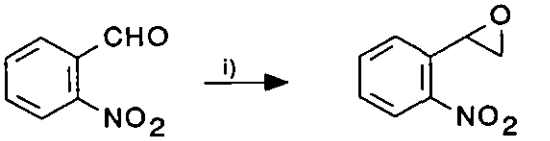
Dimethylsulfonium methylide (**65**), a heat labile reagent prepared in solution by the dehydrohalogenation of trimethylsulfonium iodide, bromide or perchlorate, is an exceedingly selective methylene transfer reagent and converts carbonyl compounds into oxiranes.[92,93] A wide variety of carbonyl compounds, including both simple and α,β-unsaturated ketones, react with this reagent with the exclusive formation of oxiranes, Scheme 2.23.



65

Scheme 2.23

Borredon[94] obtained *o*-nitrostyrene oxide (**66**) in 96 % yield by a one pot, phase transfer condensation reaction of the *o*-nitrobenzaldehyde (**57**) with trimethylsulfonium iodide in the presence of solid potassium hydroxide in acetonitrile, Scheme 2.24.



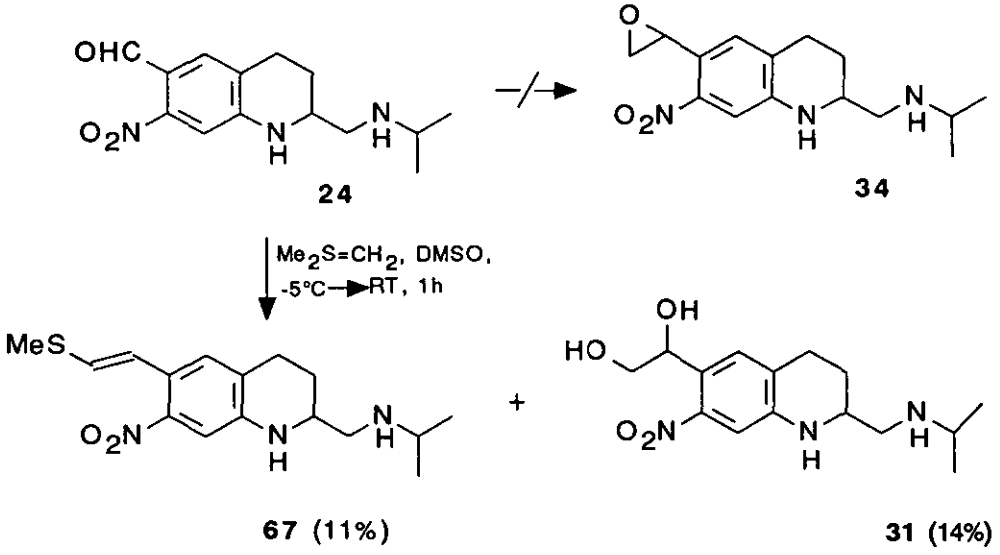
57 66 (96%)

i) Me_3SI, MeCN, H_2O, KOH, 60°C, 1h.

Scheme 2.24

Attempts to obtain oxamniquine epoxide (**34**) by this phase-transfer reaction[94] were unsuccessful. Reaction of oxamniquine aldehyde (**24**) with a molar equivalent of trimethylsulfonium iodide in aqueous acetonitrile in the presence of potassium hydroxide pellets, at 60°C for one hour yielded a mixture of products with no evidence of the desired epoxide (**34**).

The classical Corey-Chaykovsky reaction[93] of oxamniquine aldehyde (**24**) with dimethylsulfonium methylide was then investigated. Reaction of the aldehyde (**24**), in the absence of light, with 1.2 equivalents of dimethylsulfonium methylide in DMSO at -5°C for 15 minutes followed by stirring at room temperature for 45 minutes, yielded a mixture of compounds after aqueous work up and extraction with ethyl acetate. Preparative TLC on silica gel, using chloroform:ethanol (70:30) containing 1ml concentrated ammonia solution as the mobile phase, led to the isolation and characterization of two compounds. (Scheme 2.25)

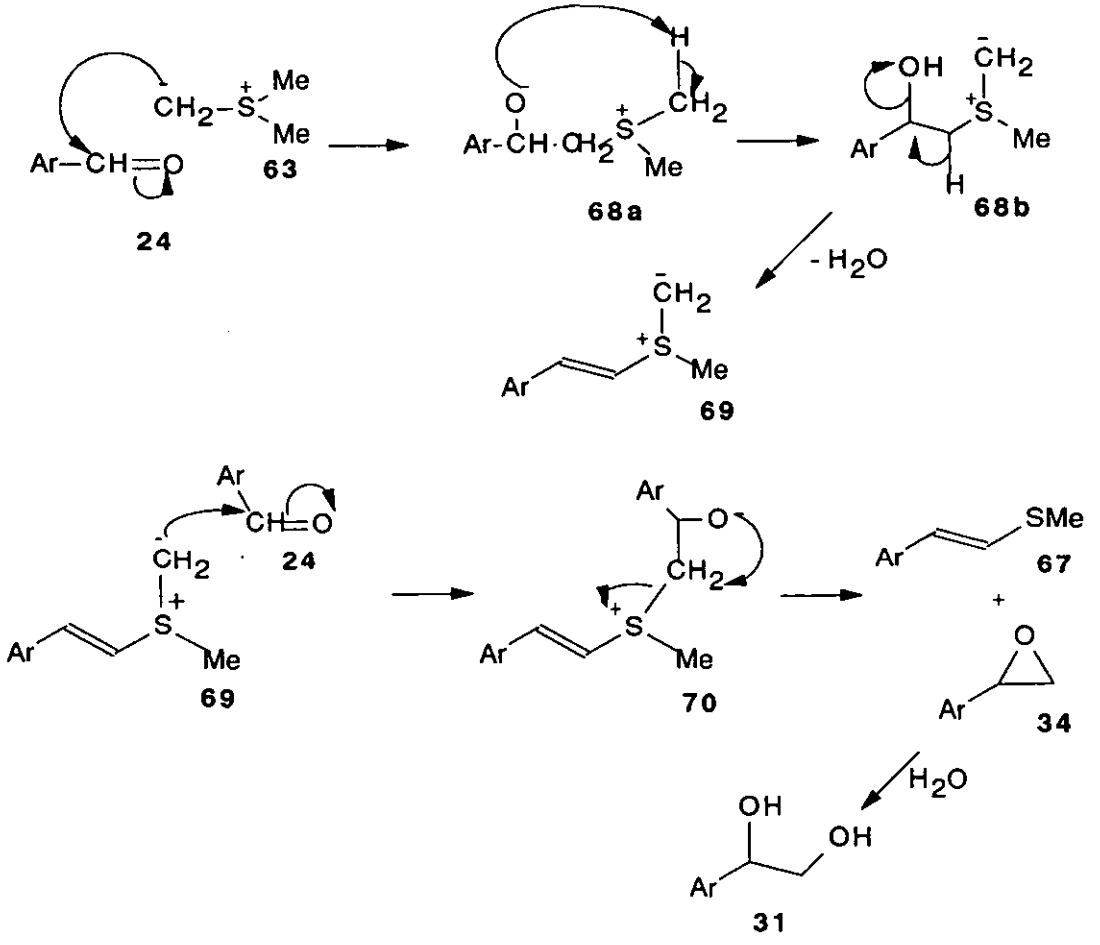


Scheme 2.25

A red coloured non polar component was isolated in 11 % yield and identified by spectroscopic methods as the methylthio vinyl derivative (**67**). The proton NMR spectrum showed the presence of a three-proton singlet at δ2.38 ppm which was assigned as the methyl protons of the thiomethyl group attached to 10-C. Two *trans*-coupled doublets with a coupling constant 17 Hz, each integrating to one proton, at δ6.58 and 6.72 ppm were assigned as the olefinic protons at 10-C and 9-C.

Chemical ionization mass spectrometry confirmed the measured mass 322.1589 consistent with the (M+H) peak from the molecular formula $C_{16}H_{24}N_3O_2S$.

The isolation of a vinyl methylthio ether from the reaction of a carbonyl compound with dimethylsulfonium methylide has not previously been documented. A possible mechanism proposed to account for the isolation of the methylthio vinyl derivative (**67**) is presented in Scheme 2.26. In this mechanism the vinyl thioether derivative (**67**) may arise via an intramolecular rearrangement and subsequent dehydration of the ylid intermediates (**68a**) and (**68b**) respectively to the ylid (**69**) which then reacts with a second molecule of the aldehyde (**24**).



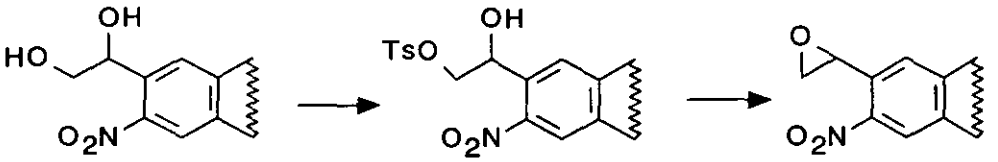
Scheme 2.26

The second component, a polar yellow compound, was isolated in 14 % yield. Spectroscopic analysis of this compound showed it to be the diol (**31**). The IR spectrum showed strong absorption peaks at 3560 cm^{-1} and 1070 cm^{-1} representing OH and C-O stretching. After a deuterium oxide shake the proton NMR spectrum revealed the presence of four exchangeable protons. One proton overlapped the methyl protons of the isopropyl group centred at δ1.06-1.17 and was assigned as the proton at N^2. A broad two-proton signal at δ1.51-1.59 ppm was assigned as the protons of the hydroxy groups at 9-C and 10-C. A third D_2O-exchangeable one proton-singlet at δ5.19 ppm was assigned as the 1-N proton.

Chemical ionization mass spectrometry confirmed the measured mass 310.1767 consistent with the (M+H) peak from the molecular formula $C_{15}H_{23}N_3O_4$.

The diol (**31**) may arise as a result of hydrolysis of the epoxide (**34**) during aqueous work up of the reaction product. However, TLC of the reaction product prior to aqueous work up showed a component with properties similar to that of the diol (**31**). Perhaps the water generated in the reaction is sufficient to effect this hydrolysis.

It was envisaged that the diol (**31**) could be converted into the epoxide (**34**) via the tosyl derivative (**71**) as shown in Scheme 2.27. However, attempts to scale up the reaction to increase yields of the diol (**31**) were not successful and resulted in the formation of very polar material which could not be isolated.



31 **71** **34**

Scheme 2.27

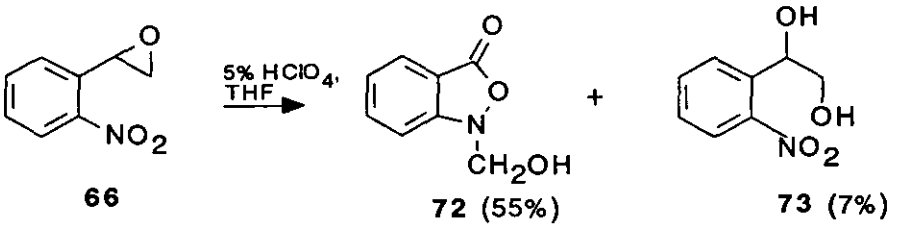
A third component of the reaction mixture, with polarity intermediate between that of the vinyl sulfide (**67**) and the diol (**31**), was noted on TLC of the crude reaction product. This component was however difficult to isolate and characterize and was thought to be the unstable epoxide (**34**).

It is possible that the stability of oxamniquine epoxide (**34**) may be influenced by the proximal *ortho* nitro group as well as the *para*-conjugated secondary amine (N_1) which deactivate and activate the system respectively.

(i) Influence of the *o*-nitro substituent on the stability of oxamniquine epoxide:
o -Nitrostyrene oxide (**66**) is known to be very photolabile and decomposes into a black mass after several months storage in the absence of light.[89] It is therefore possible that oxamniquine epoxide (**34**) may be photolabile and that this may present complications in its synthesis and isolation. Precautions were therefore taken to conduct the reaction in the absence of light and to store the reaction products in the dark and under nitrogen.

The participation of the proximate nitro group in a reaction pathway to alter the usual chemistry and stability of *o*-nitrostyrene oxide has previously been noted.

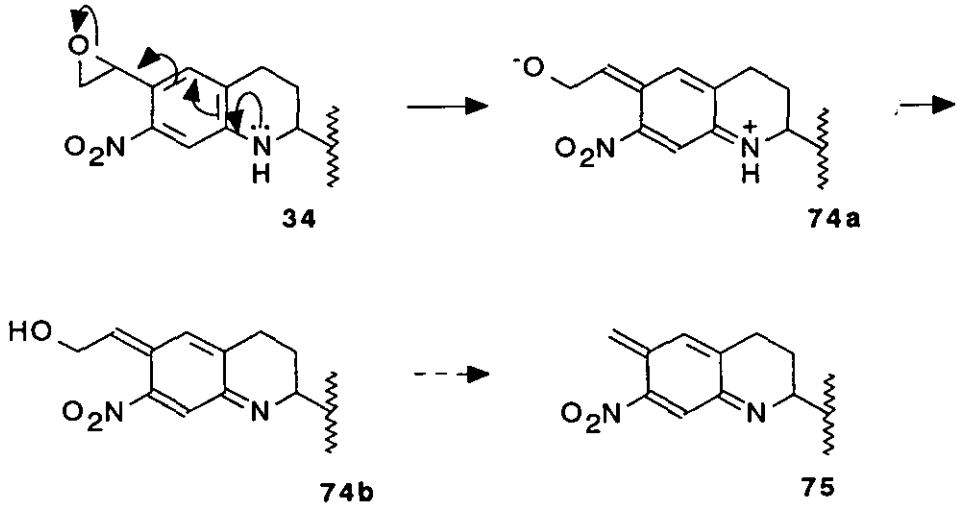
Wierenga[91] reported an acid-mediated rearrangement of *o* -nitrostyrene oxide (**66**) to 1-(hydroxymethyl)-2,1-benzisoxazol-3(1H)-one (**72**) in the process of synthesizing the photolabile protecting group (*o* -nitrophenyl)ethylene glycol (**73**). (Scheme 2.28)



Scheme 2.28

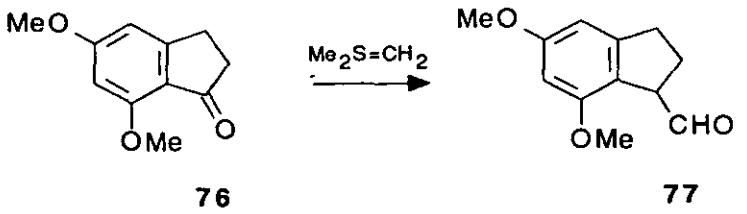
(ii) Influence of the *para*-conjugated amine on the stability of oxamniquine epoxide:

The activating influence of the *para*-conjugated amine in oxamniquine epoxide (**34**) is expected to facilitate hydrolysis/isomerisation of the epoxide as shown in Scheme 2.29. This would make isolation of the intact epoxide (**34**) even more difficult.



Scheme 2.29

The influence of conjugated electron donating groups on the synthesis and isolation of epoxides has previously been documented. Kumar[95] reported an unusual reaction of dimethylsulfonium methylide with phenyl alkyl ketones having *para* electron donating substituents in which the homologated aldehyde and not the epoxide was isolated. (Scheme 2.30)



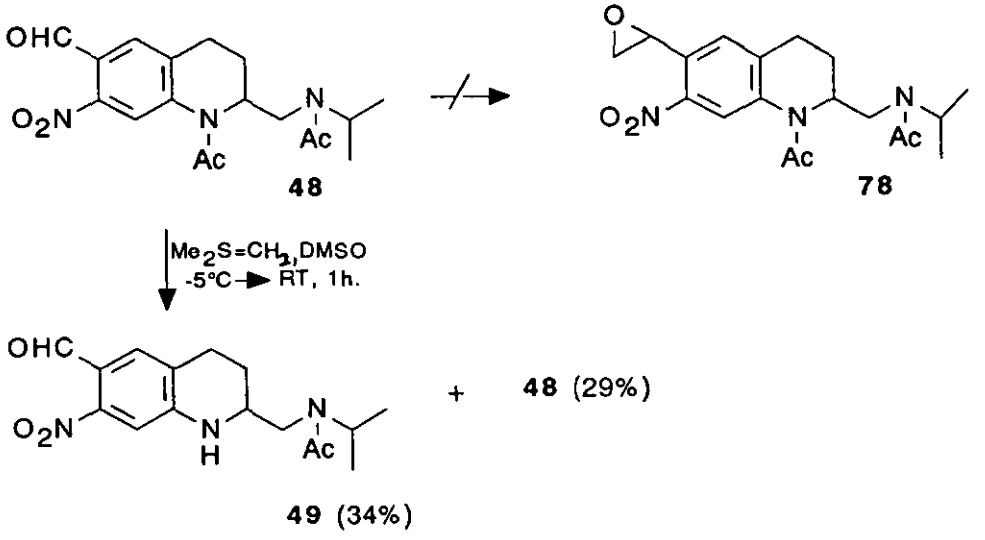
76 77

Scheme 2.30

It was therefore proposed to examine the Corey Chaykovsky reaction using the N,N-diacetyl derivative (**48**) of oxamniquine aldehyde to give the epoxide (**78**). It was anticipated that elimination of the destabilizing influence of the *para* conjugated electron-donating amine may facilitate isolation of the epoxide (**78**). This compound (**78**) was expected to be a potent alkylating agent.

2.2.8. Reaction of 6-methanoyl-1-N-acetyl-2-(N-acetyl-N-isopropylamino-methyl)-7-nitro-1,2,3,4-tetrahydroquinoline (48) with dimethylsulfonium methylide

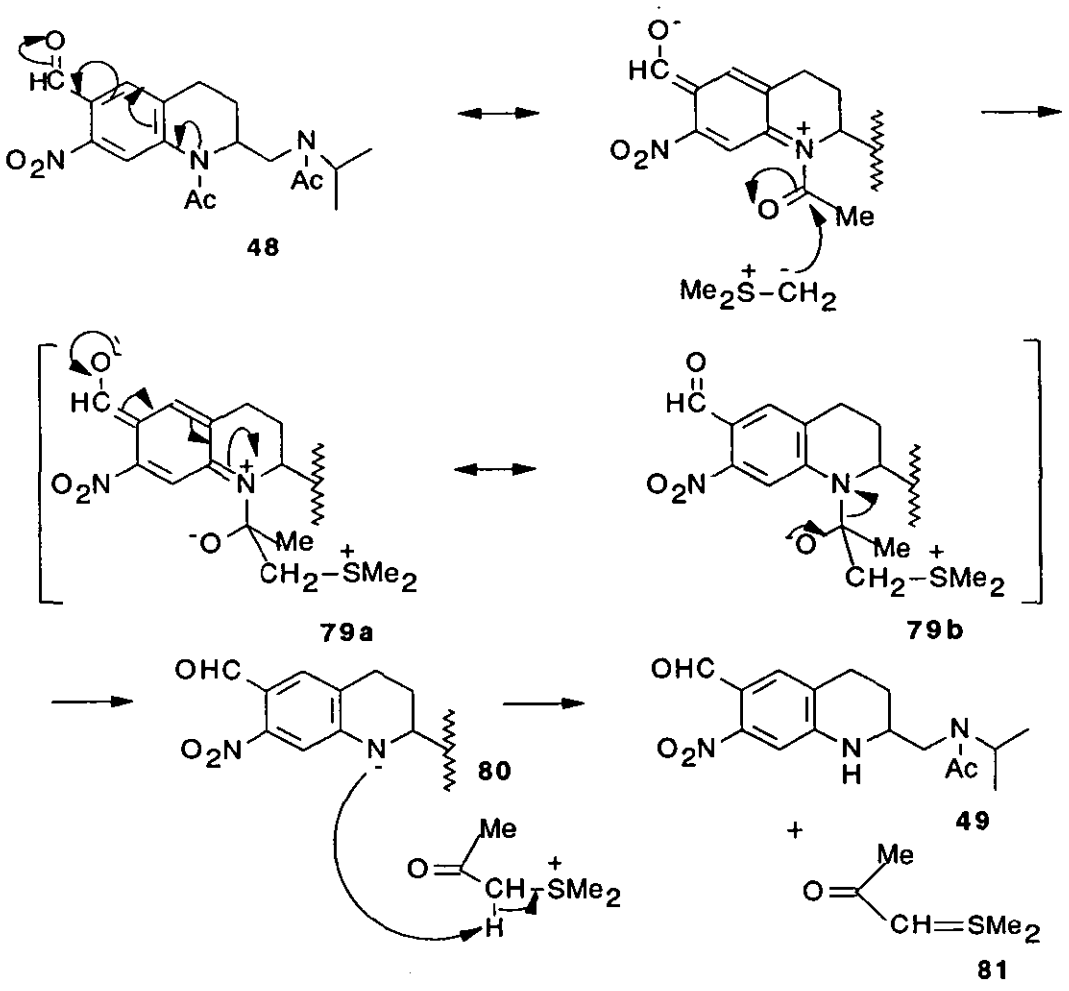
The reaction of N_1,N^2-diacetyl derivative (**48**) of oxamniquine aldehyde with dimethylsulfonium methylide was investigated. Reaction of this diacetyl derivative (**48**) with 1.2 mole equivalents of dimethylsulfonium methylide at -5°C for 15 minutes and then at room temperature for 60 minutes, followed by aqueous work up and extraction with ethyl acetate yielded a mixture of compounds. (Scheme 2.31). The desired epoxide (**78**) was not, however, isolated.



Scheme 2.31

The major component, isolated in 34 % yield, was identified as the acetyl derivative (**49**). The IR spectrum showed a carbonyl absorbance band at 1672 cm^{-1} characteristic of an aryl aldehyde and an amide carbonyl absorbance band at 1624 cm^{-1}. The proton NMR spectrum showed a three-proton singlet at δ2.22 ppm which was assigned to the methyl protons of the acetyl group. The NMR spectrum also showed a downfield shift of the protons A_1, A_2, B and 1-H as may be expected from the deshielding effects of the acetyl substituent on N^2. Two, one-proton multiplets at δ3.05-3.11 and 3.42-3.50 ppm were assigned as the protons A_2 and A_1 respectively. A one-proton multiplet at δ4.03-4.11 ppm was assigned as the proton B. A deuterium oxide-exchangeable one-proton singlet, overlapping 8-H at δ6.98 ppm, was assigned as 1-H. Electron impact mass spectrometry gave the measured molecular mass 319.1514 required for $C_{16}H_{21}N_3O_4$. Elemental analysis was consistent with this molecular formula.

The mechanism presented in Scheme 2.32 may account for the isolation of the acetyl derivative (**49**). In this mechanism preferential attack by dimethylsulfonium methylide at the amide carbonyl, at 1-N, instead of the aldehyde carbonyl at 6-C occurs.

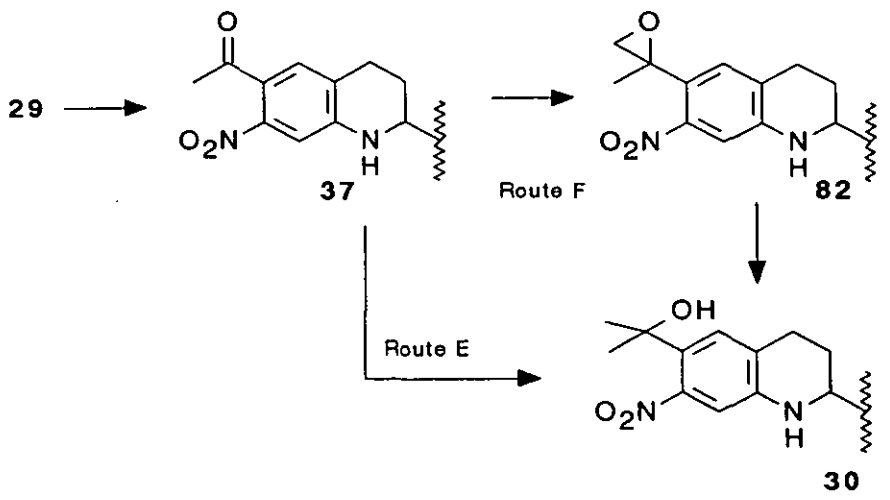


Scheme 2.32

The unreacted diacetyl derivative (**48**) was recovered in 29 % yield. A third, more polar component proved difficult to isolate and could not be characterized.

2.2.9. 6-(1,1-Dimethylhydroxymethyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine tertiary alcohol (30)

The synthetic routes proposed for the synthesis of the tertiary alcohol homologue (**30**) of oxamniquine are presented in Scheme 2.33. It was expected that oxidation of oxamniquine secondary alcohol (**29**) to the ketone (**37**) followed by reaction of this ketone (**37**) with methyl titanium trichloride would yield the tertiary alcohol (**30**) as shown by Route E. An alternative synthetic route, Route F, via the reaction of the methyl ketone (**37**) with the methylene transfer reagent, dimethylsulfonium methylide, followed by hydrolysis of the resulting epoxide (**82**) to the tertiary alcohol (**30**) was also proposed.



Scheme 2.33 Proposed routes to the synthesis of the tertiary alcohol (**30**)

(a) 6-Ethanoyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydro-quinoline, Oxamniquine methyl ketone (37)

It was proposed to prepare the ketone (**37**) by oxidation of the alcohol (**29**). We investigated the oxidation of the alcohol (**29**) using manganese dioxide, Jones reagent and the Oppenauer oxidation reaction in attempt to optimize the yield of the ketone (**37**).

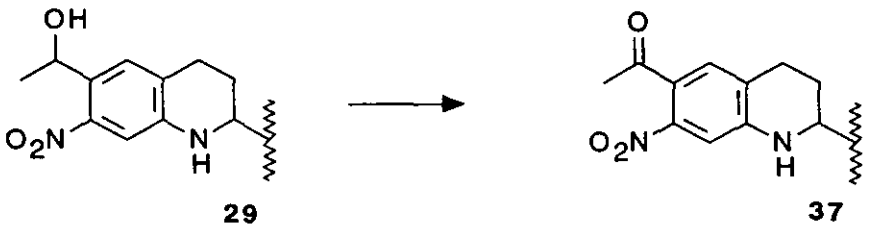
(i) Manganese dioxide oxidation

The oxidation of benzylic alcohols and structurally related alcohols by manganese dioxide is well established. Good conversions of α-substituted benzylic alcohols *e.g.* 1-phenylethanol and other substituted benzylic alcohols to the corresponding ketones have been reported.[65] We examined the reaction of the secondary alcohol (**29**) with manganese oxide at room temperature. (Scheme 2.34). Reaction of the alcohol (**29**) with 30 mole equivalents of manganese dioxide in dichloromethane, overnight at room temperature, gave a mixture of products. TLC of the crude reaction product showed the unreacted alcohol (**29**) as the major component recovered. The IR and NMR spectra of the crude product showed no evidence of the desired ketone (**37**). When the reaction time was increased to 48 hours the unreacted alcohol (**29**) was again recovered intact.

The failure to oxidize of the secondary alcohol (**29**) to the ketone (**37**) with manganese dioxide may be due to steric effects of the *ortho* nitro group. The rate of oxidation of α-substituted alcohols with manganese dioxide is known to be influenced by the steric effects of the substituents which are capable firstly, of preventing rapid adsorption of the alcohol on the surface of the active manganese dioxide and secondly, of stabilization of radicals formed in the first stage of

oxidation.[65] It is possible that preferential adsorption of the nitro group onto the active centres of manganese dioxide may hinder the adsorption of the hydroxy group and therefore precludes oxidation.

The use of higher reaction temperatures and longer reaction times was not investigated as these conditions known to be associated with loss of selectivity.[96]



29 **37**

i) MnO_2, CH_2Cl_2, RT, 17h or 48h 0%
ii) Jones reagent, acetone, 15°C, 15 min or 7h mixture **29** + **37**
iii) Al^iOPr_3, cyclohexanone, toluene, reflux, 6h 68%

Scheme 2.34

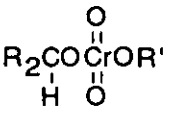
(ii) Jones oxidation

Jones reagent, 8N chromic acid, is a particularly convenient reagent for the oxidation of alcohols to the corresponding carbonyl derivative.[97] Jones reagent in acetone oxidises primary and secondary alcohols, in the presence of double or triple bonds, to aldehydes and ketones respectively without attacking the centres of unsaturation.

We investigated the reaction of the alcohol (**29**) with Jones reagent in acetone at 15°C, Scheme 2.34. In a preliminary reaction, treatment of the alcohol (**29**) with 2.4 mole equivalents of Jones reagent in acetone at 15°C for fifteen minutes, followed by basification and extraction with dichloromethane yielded a mixture of products. When the reaction was repeated under an atmosphere of nitrogen and the reaction time increased to 7 hours, a mixture was obtained.

Analysis of the IR and proton NMR spectra of the crude product revealed signals characteristic of both the alcohol (**29**) and the ketone (**37**). The IR spectrum showed a strong carbonyl absorption peak at 1672 cm^{-1} characteristic of an aryl ketone. The proton NMR spectrum showed a three-proton singlet at δ2.45 ppm which was assigned as the protons of the acetyl group. However, the unreacted alcohol (**29**) was the major component obtained and attempts to separate the ketone (**37**) and the alcohol (**29**) by chromatographic methods were not successful. The ketone was found to contain trace amounts of the alcohol (**29**) even after several chromatographic runs.

The difficulties encountered in the quantitative oxidation of the alcohol (**29**) to the ketone (**37**) with Jones reagent may be due to the presence of basic nitrogen atoms in the alcohol (**29**) as well as the steric effect of the *ortho* nitro substituent. The oxidation of hydroxyl groups to carbonyl functionalities with acidic reagents has sometimes failed in compounds containing basic nitrogen atoms, presumably because of the reaction of the amines with the acid.[98] A basic wash in the work up was therefore required to isolate the reaction products. The steric arrangement about a hydroxyl group is known to have a pronounced effect on the rate of oxidation.[97] The mechanism of chromic acid oxidation of an alcohol occurs via the reversible formation of a chromate ester intermediate (**83**) followed by cleavage of the C-H bond as the rate determining step. In highly hindered alcohols, formation of the chromate ester (**83**) becomes the rate determining step and oxidation does not occur even if the C-H bond is susceptible to cleavage.[97] It is possible that this may be true for the alcohol (**29**).



83

The reduction of orange Cr^{6+} to Cr^{3+} accompanied by the formation of a green coloured lower layer, has previously been used to monitor the progress of the oxidation reaction.[99] However, the yellow colour of the alcohol (**29**) precluded the use of this colour change as an indication of the progress of oxidation.

(iii) Oppenauer oxidation

Oppenauer involves the oxidation of an alcohol by equilibration with a carbonyl compound using an alkoxide to promote the equilibration. This reaction has been widely used in steroid chemistry although other secondary alcohols including nitrogen containing alcohols have also been oxidized.[100] Oppenauer oxidation has previously been used to oxidize secondary alcohols, poorly oxidizable with other oxidizing agents, to the corresponding ketones in good yields.

The reaction of the one mole of the alcohol (**29**) with 0.5 mole equivalents of aluminium isopropoxide in refluxing toluene, for six hours, using cyclohexanone as the hydride acceptor gave the desired ketone (**37**), Scheme 2.34. The ketone (**37**) was isolated in 68 % yield after chromatographic separation on a silica gel column using chloroform:ethanol (95:5) as the eluent. The IR spectrum showed a strong carbonyl absorbance band at 1674 cm^{-1} due to the aryl ketone. The proton NMR spectrum showed a three-proton singlet at δ2.46 ppm which was assigned as the methyl protons of the acetyl group. The proton, 1-H, was observed to be

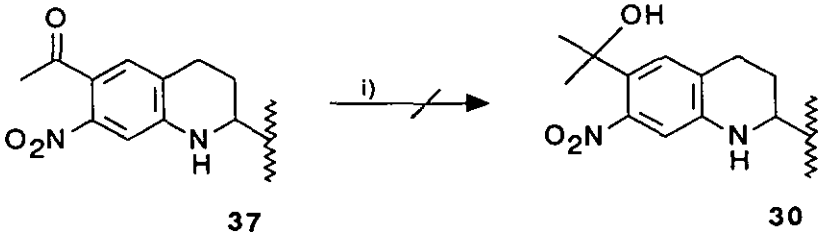
shifted downfield from δ4.98 ppm in the alcohol (**29**) to δ5.64 ppm. Electron impact mass spectrometry gave the measured molecular mass 291.1573 which was consistent with the calculated molecular mass 291.1583 required for $C_{15}H_{21}N_3O_3$.

(b) Attempted conversion of oxamniquine methyl ketone (38) to 6-(1,1-dimethyl hydroxymethyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4,-tetrahydroquinoline (30)

It was anticipated that the reaction of the ketone (**37**) with Grignard-type reagents would yield the tertiary alcohol (**30**). Notwithstanding the poor yields of the alcohol (**29**) obtained by reaction of the aldehyde (**24**) with methyl titanium trichloride and the fact that ketones are generally less susceptible than aldehydes to attack by Grignard and alkyl lithium reagents and often fail to give acceptable yields of addition products,[84] it was considered important to examine the reaction of the ketone (**37**) with Grignard-type reagents.

(i) Reaction with methyl titanium trichloride

As noted previously, Reetz[85] prepared the *para* -nitro carbinol (**62**) in 80 percent yield by reacting molar equivalents of *p* -nitroacetophenone (**61**) and methyl titanium trichloride (**56**), in diethyl ether at 0°C for three hours, Scheme 2.18. In our case, the reaction of an ethereal solution of the ketone (**37**) with five mole equivalents of methyl titanium trichloride, under nitrogen, for four hours at 0°C yielded the unchanged ketone (**37**), Scheme 2.35. The unreacted ketone (**37**) was recovered intact even when the reaction was repeated using five molar equivalents of the reagent (**56**) and stirred overnight at 10°C.



37 **30**

i) either $MeTiCl_3$, Et_2O, 0°C, 4h
or $MeTiCl_3$, Et_2O, 10°C, 19h
or 3 equiv. MeLi, -15°C, 4h
or 4 equiv. MeLi, RT, 4h

Scheme 2.35

(ii) Reaction with methyl lithium

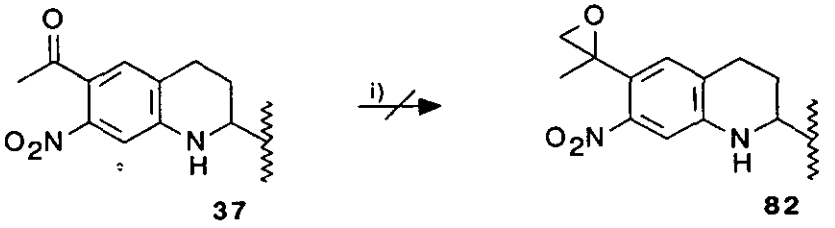
Reaction of the ketone (**37**) with methyl lithium in anhydrous conditions under nitrogen was also investigated. Initial reaction of the ketone (**37**) with three equivalents of methyl lithium at -15°C for four hours yielded the unreacted ketone (**37**), Scheme 2.35. The use of more drastic conditions:- four mole equivalents of methyl lithium at room temperature for four hours gave a dark-coloured product. Neither the ketone (**37**) nor the desired alcohol (**30**) were detectable in the reaction product. Further characterization of the product was not attempted as it was apparent that the use of these forcing conditions probably led to the destruction of the molecule.

The failure to prepare the tertiary alcohol (**30**) from the ketone (**37**) by Grignard-type reactions, using methyl titanium trichloride and methyl lithium, may be due to steric hindrance to attack on the carbonyl carbon (9-C). It is possible that the *ortho* nitro group sterically hinders the carbonyl group and makes the ketone (**37**) particularly unreactive to nucleophilic attack.

Since the attempts to synthesize the tertiary alcohol (**30**) from the ketone (**37**) by Grignard-type reactions was unsuccessful, attention was focused on the alternative synthetic pathway via the epoxide (**82**), Route F in Scheme 2.33.

(c) Attempted conversion of oxamniquine methyl ketone (37) to 6-(2-methy-oxiran-2-yl)-2-N-isopropylaminomethyl-7-nitro-tetrahydroquinoline (82)

Dimethylsulfonium methylide (**65**) is widely used as a selective methylene transfer reagent for the conversion of carbonyl compounds into oxiranes. Although our attempts to synthesize oxamniquine epoxide (**34**) from the aldehyde (**24**) using dimethylsulfonium methylide were unsuccessful we decided to investigate the reaction of the ketone (**37**) with this reagent according to the classical Corey-Chaykovsky reaction,[93] as shown in Scheme 2.36.



37 **82**

i) $Me_2S{=}CH_2$, -5°C → RT, 1h

Scheme 2.36

Reaction of the ketone (**37**) with 1.2 equivalents of dimethylsulfonium methylide in DMSO at -5°C for 15 minutes followed by stirring at room temperature for 45 minutes and normal aqueous work up yielded the unreacted ketone (**37**) as a brown film. Spectroscopic investigation of the reaction product confirmed the recovery of the ketone (**37**). The IR spectrum showed strong carbonyl absorption at 1675 cm^{-1} due to the aryl ketone. A three-proton singlet at δ2.42 ppm, was observed in the proton NMR spectrum and was assigned as the protons of the acetyl group at 9-C.

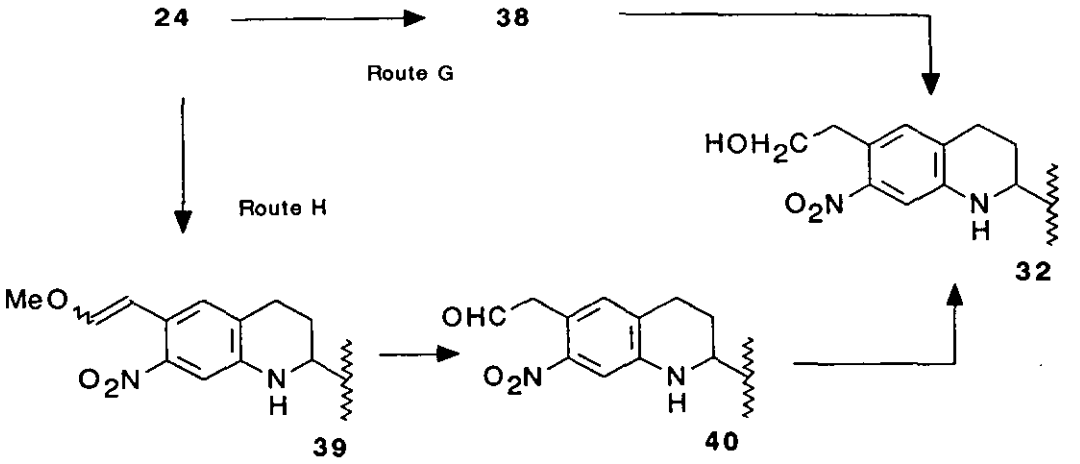
The unreacted ketone (**37**) was recovered even when the reaction conditions were modified by reacting the ketone (**37**) with four equivalents of dimethylsulfonium methylide at -5°C for one hour followed by one hour at room temperature. There was no evidence to suggest the presence of even trace quantities of the desired epoxide (**82**).

The carbonyl in the ketone (**37**) may be highly sterically hindered due to the presence of the *ortho* nitro susbtituent. It is possible that this may hinder the attack on the carbonyl by the dimethylsulfonium methylide and subsequent methylene transfer to give the epoxide (**82**).

Steric hindrance of the carbonyl at 9-C may therefore account for the resistance of this ketone (**37**) to nucleophilic addition reactions as observed in the attempts to prepare the tertiary alcohol (**30**) and the epoxide (**82**). No further attempts to synthesize the alcohol (**30**) from the ketone (**37**) were made.

2.2.10. 6-Hydroxyethyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine alcohol homologue (32)

The synthetic routes initially proposed for the synthesis of the homologous alcohol (**32**) are as shown in Scheme 2.37.



Scheme 2.37 Proposed routes to the synthesis of homologous alcohol (32)

It was proposed to synthesize the homologous alcohol (**32**) either by Route G which would involve hydroboration of the olefin (**38**) or by Route H, carbonyl olefination followed by hydrolysis[101] to give the aldehyde (**40**) and subsequent reduction to the alcohol (**32**).

Hydroboration-oxidation provides a valuable procedure for the anti-Markovnikov hydration of carbon-carbon multiple bonds.[102] The addition of the boron-hydrogen bond to carbon-carbon multiple bonds of unsaturated organic derivatives gives the organoboranes which are rapidly and essentially quantitatively oxidized with alkaline hydrogen peroxide to the corresponding anti-Markovnikov alcohols. Hydroboration of the olefin (**38**) was however, reserved as a second line approach due to the influence of the *ortho* nitro substituent. This substituent was thought to deactivate the olefin (**38**) to electrophilic attack, Scheme 2.14, as well as to sterically hinder attack on the double bond. Preparation of the alcohol (**32**) via carbonyl olefination was therefore considered a more promising route.

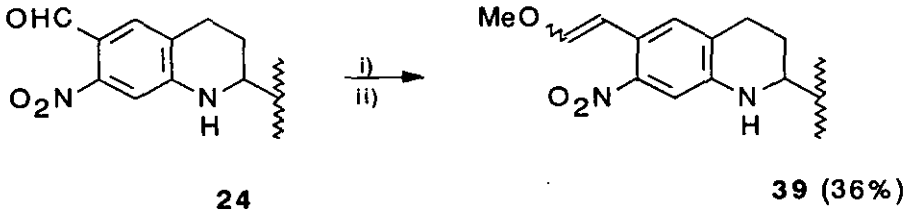
(a) 6-(2-Methoxyethenyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine methyl vinyl ether (39)

The reaction of carbonyl compounds with methoxymethylenetriphenyl phosphorane generally affords reasonable yields of the corresponding enol ethers which may be converted to the corresponding aldehyde by perchloric acid-catalyzed hydrolysis.[101]

It was proposed to synthesize the alcohol (**32**) via the Wittig-Horner reaction of the aldehyde (**24**) to yield the vinyl ether (**39**) which after acid hydrolysis would

yield the homologous aldehyde (**40**). Reduction or oxidation of this aldehyde (**40**) would yield the alcohol (**32**) and the acid (**33**) respectively.

Oxamniquine methyl vinyl ether (**39**) was prepared in 36 % yield by the Wittig-Horner reaction,[103] as shown in Scheme 2.38.



24 **39** (36%)

i) $Ph_2P(O)CH_2OMe$, LDA, THF, -78°C→RT, 20h
ii) NaH, THF, RT, 20h

Scheme 2.38

The reaction of one equivalent of the aldehyde (**24**) with two mole equivalents of the lithium derivative of diphenyl(methoxymethyl)phosphine oxide at -78°C followed by overnight stirring at room temperature yielded a red adduct. Treatment of a THF solution of the crude adduct with three equivalents of sodium hydride, at room temperature for 20 hours, yielded the vinyl ether (**39**) as a red tar. A mixture of the *cis* and *trans* isomers, in approximately 50 % relative yield, was obtained after column chromatography of the reaction product on silica gel using chloroform:ethanol (90:10) as the eluent.

The proton NMR spectrum revealed signals characteristic of a pair of *cis-trans* isomers of the vinyl ether (**39**). A pair of singlets intergrating to a total of three protons at δ3.68 and 3.73 ppm were assigned as protons of the vinylic methoxy group at 10-C. AB systems characteristic of a pair of *cis* and *trans* isomers of an olefin were also evident. Doublets centred at δ5.59, coupling constant 10 Hz, and δ6.09 ppm, coupling constant 10 Hz, each intergrating to half a proton, were assigned as the olefinic protons at 10-C and 9-C for the *cis* isomer of the vinyl ether. Doublets centred at δ6.31, coupling constant 13 Hz, and δ6.83 ppm, coupling constant 13 Hz, each integrating to half a proton were assigned as the olefinic protons at 10-C and 9-C for the *trans* isomer. Electron impact mass spectrometry gave the measured molecular mass 305.1693 which was consistent with the calculated mass 305.1739 required for $C_{16}H_{23}N_3O_3$.

Separation of the isomeric mix of vinyl ethers was not attempted since it was proposed to hydrolyse the vinyl ether (**39**) to the aldehyde (**40**).

(b) Attempted hydrolysis of the vinyl ether (39)

Carbonyl olefination followed by hydrolysis is a reliable method for aldehyde synthesis since the hydrolysis of vinyl ethers is reported to be very facile.[103] Most vinyl ethers are quantitatively converted into the corresponding aldehyde or ketone by dilute acid hydrolysis. Methyl vinyl ethers are readily hydrolysed in dilute aqueous acid conditions to give methanol and the aldehyde or ketone.

We therefore examined the acid hydrolysis of the vinyl ether (**39**) in attempt to obtain the homologous aldehyde (**40**), a key synthetic intermediate for the synthesis of the alcohol (**32**) and the acid (**33**).

(i) Reaction with *p* -toluenesulfonic acid.
In a preliminary reaction, the vinyl ether (**39**) was treated with three mole equivalents of *p*-toluenesulfonic acid in aqueous THF at room temperature for one hour. The ether (**39**) was recovered intact. The vinyl ether (**39**) was recovered unchanged even when the proportion of the acid was increased to nine equivalents and the reaction time to six hours.

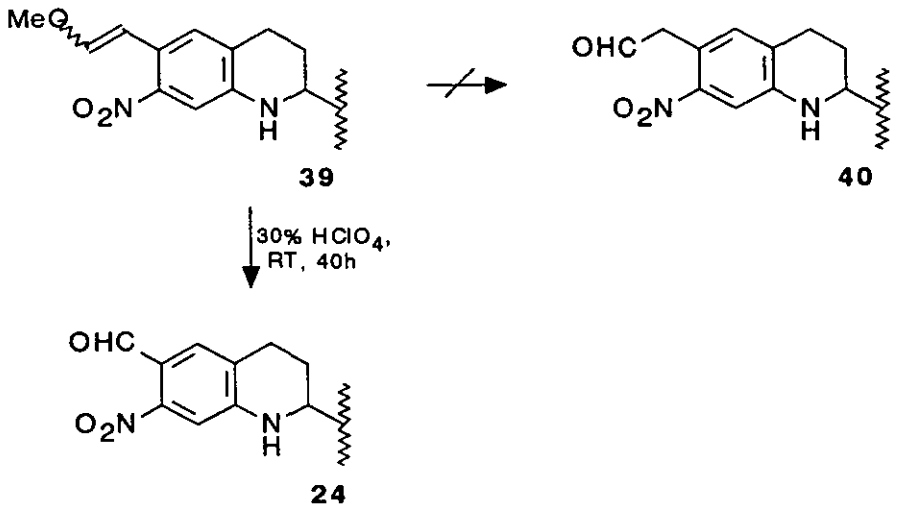
(ii) Reaction with hydrochloric acid
The reaction of the vinyl ether (**39**) with 1M hydrochloric acid in methanol, overnight, at room temperature followed by basic work-up yielded a mixture of products. The IR and proton NMR spectra of the crude reaction product did not show any signals characteristic of either the desired aldehyde (**40**) or the unreacted vinyl ether (**39**). Attempts to separate and identify the reaction products were not pursued as it was evident that the molecule did not survive the effects of hydrochloric acid.

(iii) Reaction with 30% perchloric acid

The reaction of an aqueous THF solution of the vinyl ether (**39**) with 30 % perchloric acid, at room temperature, for three hours yielded a mixture of an aldehyde product and the vinyl ether (**39**). The IR spectrum of the crude product showed a strong carbonyl absorbance band at 1673 cm^{-1}. The proton NMR spectrum showed a one-proton singlet at δ10.12 ppm which was assigned as an aryl aldehyde proton. These signals were similar to those obtained for the aldehyde (**24**).

The reaction was repeated at room temperature using 30 % perchloric acid and the reaction time increased to 48 hours. The aldehyde (**24**), 35 % yield, was obtained after preparative TLC, Scheme 2.39. Trace quantities of the unreacted vinyl ether

(**39**) were also recovered. No signals characteristic of the homologous aldehyde (**40**) were detectable even in the crude reaction product. It was expected that the IR spectrum of the aldehyde (**40**) would show carbonyl absorbance at approximately 1720 cm^{-1} while the proton NMR spectrum would show a one-proton triplet centred at approximately δ9.7 ppm.



Scheme 2.39

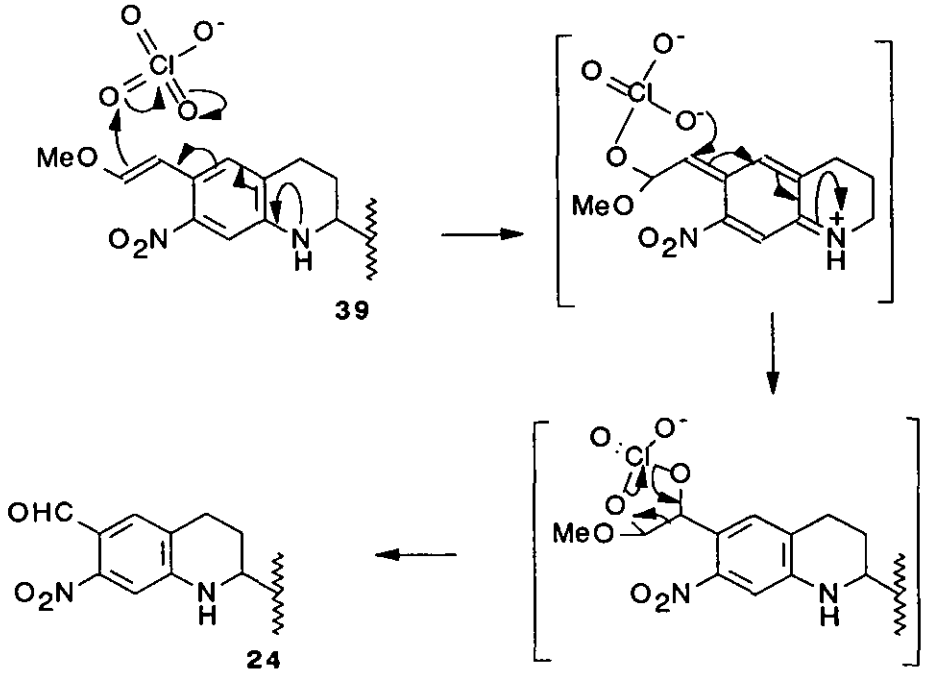
The structure of the reaction product was ascertained by spectroscopic methods. The IR spectrum showed the presence of a strong carbonyl absorbance band at 1673 cm^{-1} characteristic of an aryl conjugated aldehyde. The proton NMR spectrum showed signals characteristic of the aldehyde (**24**). Two, one-proton singlets, at δ6.99 and 7.64 ppm were assigned as the aromatic protons at 8-C and 5-H respectively. A third, one-proton singlet at δ10.12 ppm was assigned as the aldehyde group at 6-C. Electron impact mass spectrometry gave the measured molecular mass 277.1431. This was consistent with the calculated mass 277.1426 required for $C_{14}H_{19}N_3O_3$, the aldehyde (**24**). However, the aldehyde product was unstable and attempts to recrystallize it from dichloromethane-hexane only yielded a tar. The melting point was therefore not determined.

Although simple vinyl ethers are reported to be readily hydrolysed by aqueous acids to give alcohol and aldehyde or ketone, vinyl ethers in which the carbon-carbon double bond is conjugated with a double bond stabilizing group were shown to be very unreactive to acid hydrolysis.[104,105] *cis*-β-Phenylvinyl methyl ether, *trans*-β-(*p*-nitrophenyl)vinyl methyl ether and *trans*-β-cyanovinyl ethyl ether proved too unreactive to be hydrolysed at convenient rates in the dilute aqueous

acid solutions, normally used for the hydrolysis of non-conjugated vinyl ethers, and required the use of concentrated perchloric acid in the range 10-55 wt%.[104]. In agreement with these observations, the vinyl ether (**39**) was resistant to acid hydrolysis and required prolonged treatment with 30 % perchloric acid before any hydrolysis was evident.

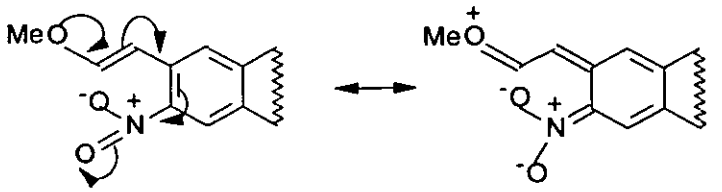
However, although Martin[101] reported that deleterious side reactions are known to occur during the hydrolysis of methyl vinyl ethers in the presence of acid-sensitive groups, the oxidative cleavage of vinyl ethers to aldehydes by perchloric does not seem to have been previously reported. It is possible that both the *para*-conjugated amine, 1-N, and the proximal *ortho* nitro substituent may play a role in the oxidative cleavage of the vinyl ether (**40**) by perchloric acid to yield the aldehyde (**24**).

A possible explanation for the oxidative cleavage of the vinyl ether (**39**) to the aldehyde (**24**), involving the influence of the *para*-conjugated amine, is illustrated by the mechanism presented in Scheme 2.40.



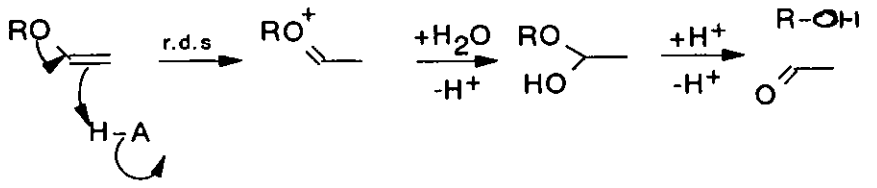
Scheme 2.40

The nitro group is a powerful electron acceptor and would be expected to interact strongly with the ether oxygen atom, a good electron donor, setting up a favourable conjugated system involving the carbon-carbon double bond as shown in Scheme 2.41.[105]



Scheme 2.41

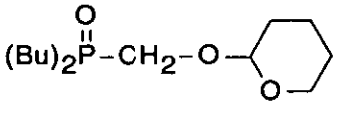
It is possible that this double bond stabilization may alter the normal mechanism of vinyl ether hydrolysis which is shown in Scheme 2.42.



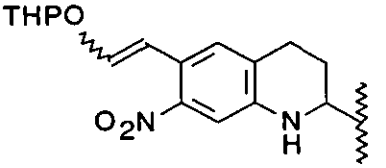
r.d.s = rate determining step

Scheme 2.42

The use of the reagent dibutylpyranyloxymethyl phosphine oxide (**84**)[106] to incorporate the tetrahydropyranyloxy (THP) function in the vinyl ether, which allows facile vinyl ether hydrolysis, has been advocated as the reagent of choice for aldehyde homologation.[107] It would be interesting to investigate the reaction of the aldehyde (**24**) with the reagent (**84**) to produce the tetrahydropyranyloxy vinyl ether (**85**). It would be interesting to note whether oxidative cleavage of the THP-vinyl ether (**85**) occurs under the milder acidic conditions, 0.2M hydrochloric acid in THF at room temperature and under argon, used for hydrolysis.



84

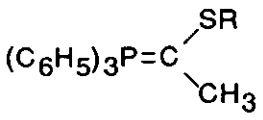


85

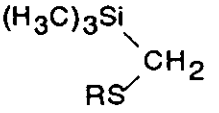
Although acid hydrolysis of the vinyl ether (**39**) did not yield the desired aldehyde (**40**), the unexpected isolation of the methylthio vinyl derivative (**67**) from the reaction of oxamniquine aldehyde (**24**) with dimethylsulfonium methylide, using the classical Corey Chaykovsky procedure, Section 2.2.7 (b), provided an alternative intermediate for the synthesis of this aldehyde (**40**).

(c) Attempted hydrolysis 6-(2-methylthio-1-ethenyl)-2-N-isopropyl-aminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, (67)

Conventional procedures for the preparation of vinyl sulfides from carbonyl compounds include (i) the application of the Wittig and Wittig-Horner reactions by the treatment of aldehydes with either alkylthio- or phenylthioalkylidenephosphoranes (**86**) and (**87**) respectively,[101] (ii) the treatment of carbonyl compounds with the lithium salts of (alkylthio) or (arylthio)(trimethylsilyl)methane (**88**) and (**89**) respectively,[108] and (iii) the reaction of carbonyl compounds with thiols in the presence of titanium tetrachloride and triethylamine.[109]

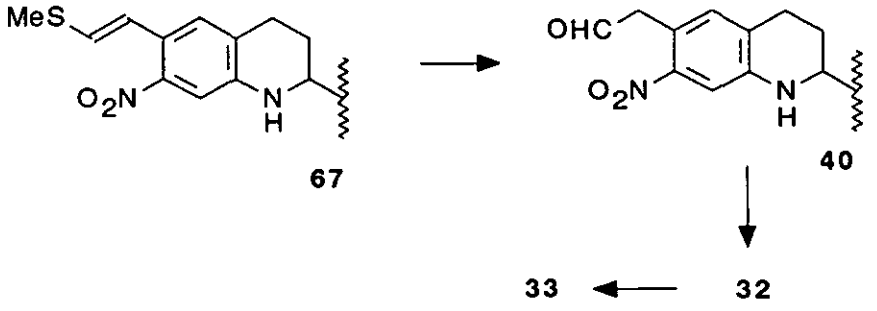


86 R = $^{n}C_4H_9$
87 R = C_6H_5



88 R = CH_3
89 R = C_6H_5

Like vinyl ethers, vinyl sulfides are latent carbonyl compounds and subsequent unmasking of the carbonyl by the appropriate hydrolytic procedure affords the aldehyde homologue of the original aldehyde.[101] It was therefore anticipated that acid hydrolysis of the methylthio vinyl derivative (**67**) would give rise to the desired aldehyde(**40**) and therefore entry into the series of the one carbon homologues of oxamniquine, the alcohol (**32**) and the acid (**33**). (Scheme 2.43).



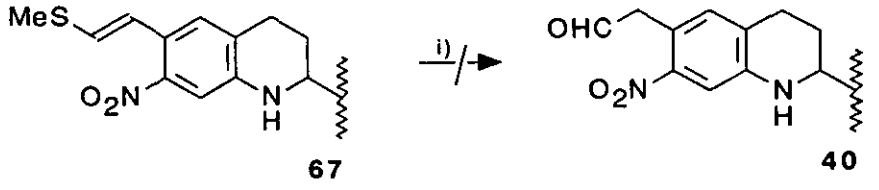
Scheme 2 .43

Although it has been reported that the major disadvantage in the use of vinyl sulfides as carbonyl equivalents is the difficulty that may be encountered on their subsequent hydrolysis to aldehydes and ketones in the presence of other functional groups, a number of mild hydrolysis methods are however, available.[101]

(i) Mercuric salt-induced hydrolysis

Corey[110] reported the mild mercuric salt-induced hydrolysis of vinyl sulfides to aldehydes and ketones in aqueous acetonitrile. We examined the reaction of the vinyl thioether (**67**) with mercuric chloride in aqueous acetonitrile.

The reaction of the methylthio vinyl derivative (**67**), dissolved in acetonitrile:water (3:1), with 2.2 mole equivalents of mercuric chloride in the same solvent at reflux temperature for 40 hours yielded the unreacted methylthio vinyl derivative (**67**). (Scheme 2.44).



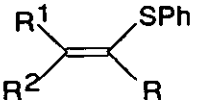
67 **40**

i) $HgCl_2$, $MeCN:H_2O$ (3:1) 65h RT or 40h reflux

Scheme 2.44

The methylthio vinyl derivative (**67**) had been recovered intact in a preliminary reaction with mercuric chloride at room temperature for 65 hours.

The success of the mercuric salt induced hydrolysis of vinyl sulfides is reported to be highly dependent on the structure of the sulfide.[111] Although mercuric salt induced hydrolysis of vinyl thioethers of ketones (**90**) gives good yields of the homologous ketones, the hydrolysis of a number of vinyl thioether derivatives of aldehydes (**91**) has been reported to be unsuccessful.[111] The unreacted starting material was recovered even after refluxing the vinyl thioether derivatives (**91**) with mercuric chloride in a (3:1) acetonitrile:water mixture for 3 days.[111] The failure to hydrolyse the methylthio vinyl derivative (**67**) by the one step reaction with mercuric chloride was therefore not too surprising.

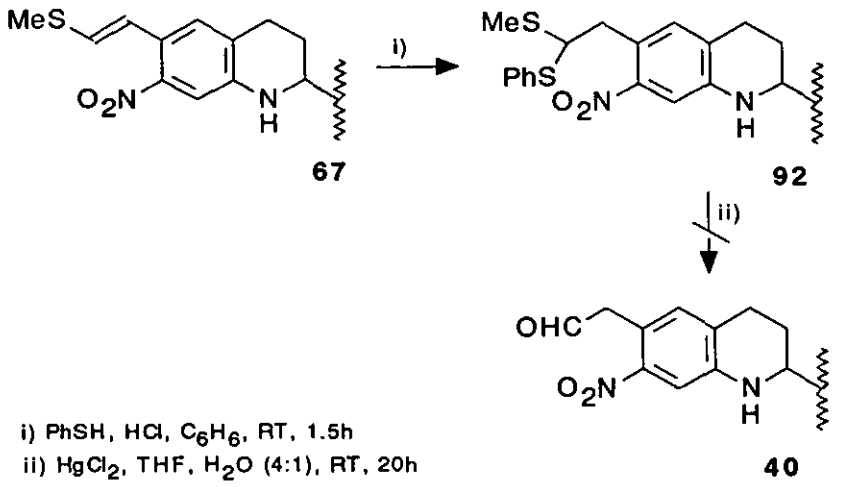


90 R = alkyl or aryl
91 R = H

(ii) Vinyl sulfide hydrolysis via the thioacetal

It has been reported that the difficulty encountered in the one step hydrolysis of vinyl thioethers may be circumvented by the addition of a thiol to the vinyl thioether to form the thioacetal which is subsequently hydrolysed to the aldehyde

by mercuric chloride induced hydrolysis.[111,112] We attempted to hydrolyse the vinyl thioether (**67**) by addition of thiophenol to the double bond to form the thioacetal (**92**) followed by hydrolysis with mercuric chloride as shown in Scheme 2.45.



67 **92** **40**

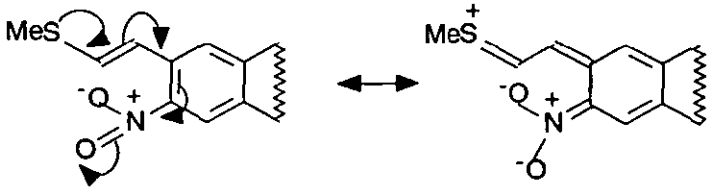
i) PhSH, HCl, C_6H_6, RT, 1.5h
ii) $HgCl_2$, THF, H_2O (4:1), RT, 20h

Scheme 2.45

The reaction of the vinyl thioether (**67**) with excess thiophenol, in hydrogen chloride saturated benzene, at room temperature for 1.5 hours, followed by removal of the solvent yielded a red adduct. Treatment of this adduct with two mole equivalents of mercuric chloride in THF: water (4:1) overnight, at room temperature, yielded a red film after basic work up. TLC of the crude product showed a mixture of compounds. The proton NMR spectrum of the reaction product was consistent with that for the methylthio vinyl derivative (**67**). A three-proton singlet at δ2.34 ppm was assigned as the protons of the thiomethyl substituent at 10-C. Two one-proton doublets at δ6.58 ppm, coupling constant 16.9 Hz, and δ6.75 ppm, coupling constant 16.9 Hz, were assigned as the olefinic protons 10-H and 9-H. A two-proton singlet at δ7.06 ppm was assigned as the two aromatic protons 8-H and 5-H. The IR spectrum did not show any carbonyl absorbance bands. The unreacted methylthio vinyl derivative (**67**) was the major component of the reaction product.

Hydrogen chloride has been reported to catalyse the addition of thiophenol to vinyl thioethers to form the corresponding thioacetals which are highly susceptible to mercuric chloride induced hydrolysis.[111] This however, was not observed with the methylthio vinyl derivative (**67**). It is possible that the failure to hydrolyse the methylthio vinyl derivative (**67**) to the aldehyde (**40**) may be attributed to the steric nature of the vinyl derivative (**67**) as well as to the

deactivating influence of the *ortho*-conjugated nitro substituent. The vinyl thioether (**67**) is analogous to the vinyl ether (**39**) and as previously noted the nitro group may stabilize the double bond by setting up a conjugated system involving the sulphur atom of the thioether and the carbon carbon double bond. (Scheme 2.46). This may be expected to decrease the reactivity of the methylthio vinyl derivative (**67**).



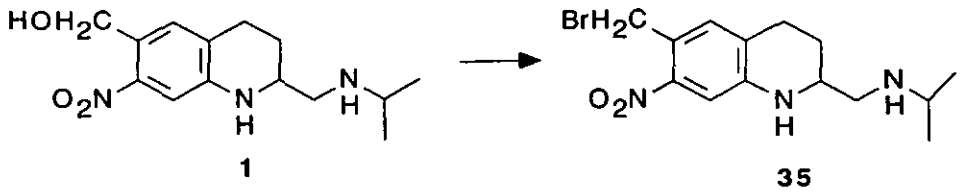
Scheme 2.46

It is therefore likely that the thioacetal derivative (**92**) was not in fact formed to a significant degree, hence the failure of the mercuric chloride induced hydrolysis of the thioacetal.

Attempts to synthesize the aldehyde (**40**) by hydrolysis of either the vinyl ether (**39**) or the vinyl thioether (**67**) were unsuccessful. The vinyl thioether (**67**) was resistant to hydrolysis and treatment of the vinyl ether (**39**) with 30 % perchloric acid resulted in oxidative cleavage to the aldehyde (**24**). The attempts to obtain the alcohol (**32**) by carbonyl olefination and hydrolysis were therefore unsuccessful. Although hydroboration of the olefin (**38**) had been considered as an alternative route for the preparation of the alcohol (**32**), Route G in Scheme 2.37, the time scale did not permit an examination of this reaction. Further attempts to prepare the homologous alcohol (**32**) were not pursued and attention was focused on attempts to prepare the bromomethyl derivative (**35**), the last in the series of the target compounds.

2.2.11. 6-Bromomethyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetra-hydroquinoline, Oxamniquine bromide (35)

Several methods are available for the conversion of benzylic alcohols to benzylic bromides.[113] In attempt to synthesize the 6-bromomethyl derivative (**35**) it was proposed to examine the reaction of oxamniquine (**1**) with three commonly used reagents: (i) carbon tetrabromide and triphenyl phosphine, (ii) hydrobromic acid and (iii) phosphorus tribromide. (Scheme 2.47).



i) CBr_4, Ph_3P, THF, RT, 1h
ii) HBr, AcOH, RT, overnight
iii) PBr_3, Et_2O, pyridine

Scheme 2.47

(a) Reaction with carbon tetrabromide and triphenyl phosphine

The method of conversion of alcohols to the corresponding alkyl halide by reaction with carbon tetrahalide and tertiary phosphines is well known. The reaction is quite general and proceeds under mild, essentially neutral conditions with little heat being required and gives good yields of the halide from primary and secondary alcohols.[114]

We investigated the reaction of oxamniquine with carbon tetrabromide in the presence of triphenyl phosphine according to the method described by Lan.[115] The reaction of a solution of oxamniquine (1) in dry THF with 1.2 mole equivalents of carbon tetrabromide, in the presence of 1.2 mole equivalents of triphenyl phosphine, for 1 hour at room temperature yielded a yellow suspension. The suspension was dissolved in ethanol and evaporated *in vacuo* to yield a yellow film which was shown by TLC to be a mixture of compounds.

Chromatographic separation on a silica gel column using chloroform:ethanol as the eluent yielded triphenyl phosphine oxide and a yellow solid. This yellow solid melted with decomposition at 270°C. The presence of an NH_2^+ absorbance band at 2403 cm^{-1} as well as an OH absorbance at 3557 cm^{-1} and C-O stretching absorbance at 1046 cm^{-1} in the IR spectrum suggested that the solid was the hydrobromide salt of oxamniquine. However, the NMR spectrum was not taken as the solid was insoluble in chloroform.

Treatment of the yellow solid with 1M sodium hydroxide and back extraction with chloroform yielded a yellow solid with TLC, IR spectroscopic and NMR spectroscopic properties similar to oxamniquine. The IR spectrum showed OH absorbance at 3620 cm^{-1}, NH absorbance at 3400 cm^{-1} and a strong C-O stretching absorbance at 1046 cm^{-1}. The proton NMR spectrum showed a singlet at δ4.69 ppm which was assigned as the benzylic methylene protons at 9-C. The aromatic protons 5-H

and 8-H appeared as singlets at δ7.10 ppm and δ7.20 ppm respectively. A deuterium oxide-exchangeable singlet at δ5.10 ppm was assigned as the proton 1-H.

It was therefore apparent that the reaction of oxamniquine with carbon tetrabromide in the presence of triphenyl phosphine had resulted in the formation of the hydrobromide salt of oxamniquine. It is however, possible that the hydrobromide salt of the bromomethyl derivative (**35**) could also have been formed but was decomposed in the presence of base so that only oxamniquine was isolated.

(b) Reaction with hydrobromic acid in acetic acid

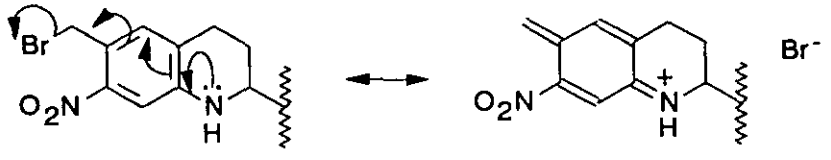
In a preliminary study the reaction of oxamniquine with hydrobromic acid in the presence of acetic acid according to a method described by Vogtle[116] was investigated. Treatment of a solution of oxamniquine in acetic acid with a cold solution of 33% hydrobromic acid, overnight at room temperature, yielded a suspension. Addition of diethyl ether deposited a white precipitate which was collected at the pump. This was identified as an amine salt by the absorbance band at 2340 cm^{-1} in the IR spectrum which is characteristic of NH^+ absorbance. Attempts to release the free base by suspending the salt in triethylamine and extracting with chloroform yielded a mixture of compounds.

(c) Reaction with phosphorus tribromide

Although the reaction of oxamniquine (**1**) with phosphorus tribromide had been proposed as one of the methods for preparing the bromomethyl derivative (**35**), the insolubility of oxamniquine in dry diethyl ether prevented the investigation of this reaction according to the methods reported in the literature.[117,118]

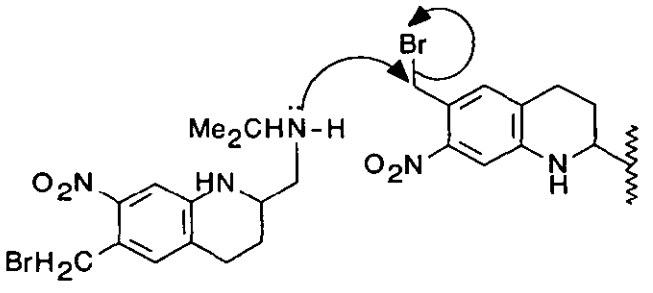
It is possible that the bromomethyl derivative (**35**) may be unstable due to the presence of basic nitrogen atoms in the oxamniquine molecule.

Delocalization of the lone pair electrons of the ring nitrogen into the aromatic ring may facilitate the elimination of the bromide ion. (Scheme 2.48).



Scheme 2.48

Since the bromide ion is a good leaving group, the intramolecular or intermolecular nucleophilic attack on 9-C by the nitrogen lone pair electrons to form an amine derivative is possible. (Scheme 2.48)



Scheme 2.48

It would be useful to isolate the reactivity of the 6-hydroxymethyl functionality by examining the reaction of the N_1,N^2 diacetyl derivative (**47**) with either carbon tetrabromide in the presence of triphenyl phosphine or hydrobromic acid or phosphorus tribromide.

In summary several novel analogues of oxamniquine were obtained by structural modification of the molecule. The 6-hydroxymethyl group of oxamniquine was replaced to yield the following derivatives:- the acid (**9**), the alcohol (**29**), the ketone (**37**), the olefin (**38**), the diol (**31**), the methylthio vinyl derivative (**67**), the methyl vinyl ether (**39**) and the arylamine (**52**). With the exception of the acid (**9**), which is the major metabolite of oxamniquine in humans, the derivatives synthesized were all novel compounds. Two novel acetyl derivatives of oxamniquine, the peracetylated derivative (**46**) and the N_1,N^2 diacetyl derivative (**47**) were prepared. In addition the novel acetyl derivatives of oxamniquine aldehyde the N_1, N^2 diacetyl derivative (**48**) and the N^2 acetyl derivative (**49**) were also prepared.

Some unusual reactions were observed. These include the oxidative cleavage of the methyl vinyl ether (**39**) with perchloric acid to give the aldehyde (**24**) and the synthesis of the methylthio vinyl derivative (**67**) and the diol (**31**) by an intramolecular ylid rearrangement in the reaction of oxamniquine with dimethylsulfonium methylide.

Of the target compounds proposed, synthesis of the acid (**9**), the alcohol (**29**), the diol (**31**) and the arylamine (**52**) was realized. The acid (**9**) and the alcohol (**29**) were screened for CNS activity in an *in vivo* model.

CHAPTER THREE

EXPERIMENTAL FOR THE PREPARATION OF OXAMNIQUINE ANALOGUES

3.1 General Information About Procedures

Solvents were purified and dried according to standard techniques as shown below. Melting points were determined on the Electrothermal Digital melting point apparatus, in open capillary tubes, and are uncorrected. For column chromatography on silica gel, Matrex®Silica 60 was used. TLC was carried out using aluminium backed plates coated with Merck Kieselgel 60 F_{254}. Elemental analyses were done at Medac Ltd., Department of Chemistry, Brunel University. Infrared spectra were recorded using either a Nicolet-205 FTIR or a Pye Unicam Infra Red Spectrophotometer integrated with a Philips IR Data Station.The spectra were recorded either as thin films or in chloroform solution. Proton NMR spectra were recorded on the Bruker 250AC Spectrometer. The samples, unless otherwise stated, were dissolved in deuteriated chloroform and tetramethylsilane (TMS) was used as the internal standard. Chemical shifts are quoted in ppm relative to TMS and coupling constants, J, are given in Hz. Mass spectra were recorded on the Kratos MS80/DC90 Spectrometer by electron impact. Chemical ionization mass spectra were recorded by the Serc Mass Spectrometry Service Centre, Chemistry Department, University College of Swansea.

3.1.1 Solvent Drying Procedures

Acetone	Refluxed with potassium permanganate then distilled.
Acetyl chloride	Refluxed over phosphorus pentachloride then distilled.
Benzene	Dried, refluxed and distilled over sodium wire then stored over sodium wire.
Chloroform	Dried with calcium chloride, refluxed with phosphorus pentachloride and distilled.
Dichloromethane	Refluxed with phosphorus pentachloride and distilled.
Diethyl ether	Refluxed with calcium chloride then distilled and stored over sodium wire. Anhydrous diethyl ether was freshly distilled after refluxing with lithium aluminium hydride.
Dimethyl sulfoxide	Dried over calcium hydride then distilled under reduced pressure. Stored over 4A molecular sieve.
Ethanol	Refluxed with and distilled from magnesium activated with iodine. Stored over 4A molecular sieves.
Ethyl acetate	Refluxed with calcium chloride and distilled.
Hexane	Refluxed with calcium chloride and distilled.
Methanol	Refluxed with and distilled from magnesium activated with iodine. Stored over 4A molecular sieves.

Nitrogen	Oxygen free, passed through silica gel.
Petroleum ether 60/80	Refluxed with calcium chloride and distilled.
Tetrahydrofuran	Dried and distilled from sodium and benzophenone until purple colour of sodium benzophenone ketyl was well established.
Toluene	Dried with calcium hydride, refluxed and distilled.

3.2 6-Hydroxymethyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4,-tetrahydro-quinoline, Oxamniquine (1)

Pure oxamniquine reference compound, UK 4271 Lot 603/OX/701, was a generous gift from Pfizer Central Research, Sandwich, Kent, England. ν_{max} ($CHCl_3$)/cm^{-1} 3388 (NH), 1618 (benzene), 1508, 1348 (conj C-NO_2) and 1162 (C-O); δ_H (250 MHz; $CDCl_3$) 1.06-1.10 (6H, 2d, J 5.5, CH<u>Me</u>$_2$), 1.47-1.63 (3H, br m, 3-H, N<u>H</u>CH, OH), 1.91-2.01 (1H, m, 3-H), 2.42-2.50 (1H, dd, J10.2 and 3.4, A_2), 2.75-2.83 (3H, m, 4-CH_2, B), 2.86-2.92 (1H, dd, J 6.8 and 3.4, A_1), 3.23-3.32 (1H, m, 2-H), 4.69 (2H, s, 9-CH_2), 5.06 (1H, s, 1-H), 7.11 (1H, s, 5-H) and 7.20 (1H, s, 8-H); m/z, CI 280.1661 (M+H, 100%), 248 (25), 72 (60), 58 (35) and 44 (15).

3.3 6-Methanoyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4,-tetra-hydroquinoline, Oxamniquine aldehyde (24)[49]

To a stirred suspension of oxamniquine (1.025g, 3.696 mmol) in dichloromethane was added manganese dioxide (3.061g, 35 mmol) in 2 portions in 15 minutes. The suspension was stirred overnight at room temperature, filtered and the filtrate evaporated to yield the aldehyde (**24**) (0.742g, 73%), as brick-red crystals, m.p. 99.3-99.8°C,(from dichloromethane-hexane) (lit.,[49] 99-100°C). ν_{max} ($CHCl_3$)/cm^{-1} 3384 (NH), 1670 (aryl aldehyde), 1602 (benzene),1520 and 1330 (conj C-NO_2); δ_H (250 MHz, $CDCl_3$) 1.10-1.25 (6H, 2d, CH<u>Me</u>$_2$), 1.47-1.57 (1H, m, 3-H), 1.82 (1H, br s, N<u>H</u>CH), 1.97-2.04 (1H, m. 3-H), 2.42-2.51 (1H, t, A_2), 2.79-2.91 (3H, m, 4-CH_2, B), 2.97-3.03 (1H, dd, A_1), 3.37-3.45 (1H, t, 2-H), 6.00 (1H, br s, 1-H), 6.99 (1H, s, 8-H), 7.63 (1H, s, 5-H) and 10.13 (1H, s, 9-C<u>H</u>O); m/z CI 278 (M+H, 100%), 248 (65) and 72 (35). (Found: C, 60.5; H 6.9; N, 15.15%; M+H, 278.1505. Calc. for $C_{14}H_{19}N_3O_3$ C, 60.9; H, 6.9; N,15.15%; M, 277.1426).

3.4 6-(1-Acetoxymethyl)-1-N-acetyl-2-(N-acetyl-N-isopropylamino-methyl)-7-nitro-1,2,3,4,-tetrahydroquinoline, Oxamniquine N_1,N^2-diacetyl-6-methylacetate ester (46)

Acetic anhydride (125ml) was added to oxamniquine (6.06g, 22 mmol). The resulting yellow solution was refluxed for 2.5 hours, poured into water (500 ml) and stirred at room temperature for 2 hours. The aqueous mixture was extracted with four volumes of chloroform, the organic extracts washed with water, dried (anhydrous magnesium sulfate) and evaporated to a small volume. The concentrate was then washed with saturated sodium hydrogen carbonate until effervescence ceased. The chloroform extract was dried (magnesium sulfate) and evaporated to yield the crude triacetylated derivative. Recrystallization from petroleum ether (60/80) : ethanol (80:20) gave the peracetylated derivative (**46**) (6.48g, 72%), as pale yellow recrystals, m.p. 134.0-134.6°C. ν_{max} (nujol)/cm^{-1} 1740 (acetate), 1660, 1634 (amide I and amide II), 1522, 1348 (conj C-NO_2) and 1052 (C-O); δ_H (250MHz, $CDCl_3$) 1.29-1.56 (6H, m, CH$\underline{Me}_2$), 1.89-1.91 (1H, m, 3-H), 2.15 (3H, s, N^2-CO$\underline{Me}$), 2.17 (3H, s, 9-CO$\underline{Me}$), 2.22 (3H, s, 1-CO$\underline{Me}$), 2.15-2.27 (1H, m, 3-H), 2.73-3.05 (3H, br m, 4-CH_2, A_2), 3.38-3.46 (1H, dd, A_1), 3.97-4.09 (1H, quintet, B), 4.92-4.99 (1H, quintet, 2-H), 5.49 (2H, s, 9-CH_2), 7.36 (1H, s, 5-H) and 8.27 (1H, br s, 8-H); m/z CI 406 (M+H, 100%), 114 (50) and 72 (92). (Found: C, 59.5; H, 6.85; N, 10.3%; M+H, 406.1980. $C_{20}H_{27}N_3O_6$ requires C, 59.25; H, 6.7; N 10.4 %; M, 405.1900).

3.5 6-Hydroxymethyl-1-N-acetyl-2-(N-acetyl-N-isopropylamino-methyl)-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine-N_1,N^2-diacetamide (47)

To a solution of the peracetylated derivative (**46**) (1.005g, 2.481 mmol), dissolved in methanol (30ml), was added 2% sodium carbonate (100ml) and the resulting solution stirred overnight at room temperature. The reaction mix was concentrated *in vacuo* and extracted with ethyl acetate. The extract was washed with water, dried (magnesium sulfate) and evaporated to yield the crude diacetate (0.782g). Recrystallization from ethanol gave the acetyl derivative (**47**) as yellow crystals (0.720g, 80%), m.p. 165.6-166.1°C. ν_{max} (nujol)/cm^{-1} 1654, 1634 (amide I and amide II), 1612 (C=C, benzene), 1512 and 1342 (conj C-NO_2); δ_H (250MHz, $CDCl_3$) 1.10-1.22 (6H, m, CH$\underline{Me}_2$), 1.87 (1H, br s, 3-H), 2.13-2.21 (1H, m, 3-H), 2.15 (3H, s, N^2-CO$\underline{Me}$), 2.21 (3H, s, 1-CO$\underline{Me}$), 2.62-3.12 (3H, m, 4-CH_2, A_2), 3.37-3.44 (1H, dd, A_1), 4.00-4.05 (1H, quintet, B), 4.94-5.00 (3H, s overlaps m, 9-CH_2, 2-H), 7.53 (1H, s, 5-H) and 8.23 (1H, br s, 8-H); m/z CI 364

(M+H, 100%), 332 (80), 102 (50) and 72 (40). (Found: C, 59.55; H, 7.0; N, 11.5%; M+H, 364.1870. $C_{18}H_{25}N_3O_5$ requires C, 59.5; H, 6.9; N, 11.6 %; M, 363.1794).

3.6 6-methanoyl-1-N-acetyl-2-(N-acetyl-N-isopropylaminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline, N_1,N^2-diacetyl oxamniquine aldehyde (48)

To a magnetically stirred solution of the diacetamide (**47**) (0.548g, 1.510mmol) in dichloromethane, was added manganese dioxide (1.642g) in 2 portions over 15 minutes. The black suspension was stirred overnight at room temperature, filtered and the filtrate evaporated to yield the crude aldehyde (0.486g). Recrystallization from dichloromethane : hexane (30:70) gave the aldehyde (**48**) as yellow crystals (0.394g, 72%), m.p. 137.1-138.6°C. ν_{max} 1686, 1628 (amide I and amide II), 1664 (aryl aldehyde), 1522 and 1340 (conj C-NO_2); δ_H (250MHz, $CDCl_3$) 1.09-1.16 (6H, 2d, CH$\underline{Me}_2$), 1.94-1.99 (1H, m, 3-H), 2.01-2.23 (1H, m, 3-H), overlaps 2.16 (3H, s, N^2-CO$\underline{Me}$), 2.27 (3H, s, 1-CO$\underline{Me}$), 2.76-3.06 (3H, m, 4-CH_2, A_2), 3.31-3.39 (1H, dd, A_1), 3.98-4.08 (1H, m, B), 4.96-5.05 (1H, m, 2-H), 7.78 (1H, s, 8-H), 8. 41 (1H, s, 5-H) and 10.38 (1H, s, 9-C$\underline{H}$O); m/z CI 362 (M+H, 50%), 332 (100) and 72 (30). (Found: C, 59.5; H, 6.25; N, 11.2%; M+H, 362.1716. $C_{18}H_{23}N_3O_5$ requires C, 59.8; H, 6.4; N,11.6%; M, 361.1638).

3.7 6-Methanoyl-2-(N-acetyl-N-isopropylaminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline, N^2-acetyl oxamniquine aldehyde (49)

Acetic anhydride (10ml) was added to the aldehyde (**24**) (0.152g, 0.549mmol). The resulting yellow solution was refluxed for 2.5 hours, cooled to room temperature, diluted with water (100ml) and stirred at room temperature for 3 hours. The aqueous reaction mix was extracted with chloroform. The extract was washed with water, dried (magnesium sulfate) and concentrated to a small volume. The concentrate was washed with saturated sodium hydrogen carbonate and water, dried (magnesium sulfate) and evaporated to yield the crude acetate. Recrystallization from ethanol gave the acetyl derivative (**49**) as a bright yellow powder (0.125g, 71%), m.p. 161.2-162.4°C. ν_{max} ($CHCl_3$)/cm^{-1} 3268 (NH), 1658 (aryl aldehyde) and 1632 (amide), 1598 (C=C, benzene), 1524 and 1348 (conj C-NO_2); δ_H (250 MHz, $CDCl_3$) 1.19-1.27 (6H, 2d, CH$\underline{Me}_2$), 1.55-1.67 (1H, br m, 3-H), 1.93-2.00 (1H, m, 3-H), 2.22 (3H, s, N^2-CO$\underline{Me}$), 2.81-2.87 (2H, m, 4-CH_2), 3.05-3.11 (1H, dd, A_2), 3.45-3.51 (1H, m, 2-H), 3.61-3.73 (1H, dd, A_1), 4.06-4.11 (1H, quintet, B), 6.98 (2H, s, 8-H, 1-H), 7,62 (1H, s, 5-H) and 10.12 (1H, s, 9-C$\underline{H}$O); m/z EI 319 (M^+, 20%), 205 (83), 115 (100) and 72 (85).

$C_{16}H_{21}N_3O_4$ requires M, 319.1532.

3.8 6-Carboxy-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetra-hydroquinoline, Oxamniquine 6 carboxylic acid (9)

Silver oxide was prepared *in situ* by the addition of a solution of silver nitrate (0.185g, 1.089mmol) in water (10ml) to stirred solution of sodium hydroxide in water (10ml). A grey semi-solid resulted. To the vigorously stirred suspension was added, with cooling, the aldehyde (**24**) (0.155g, 0.56mmol) in THF (10ml). The resultant suspension was stirred at room temperature for 18 hours and a silver mirror was deposited. The suspension was filtered through celite, washed with diethyl ether. The aqueous phase was acidified to pH1 with 2M HCl and extracted with ethyl acetate. The residual aqueous phase was evaporated as the methanol azeotrope. Charcoal decolourization of the crude residue and recrystallization from aqueous ethanol yielded the hydrochloride salt of the acid (**9**) as pale yellow crystals (0.097g, 61%), m.p. 260°C (decomp.), [lit.,[22] 271°C (decomp.)]. ν_{max} (nujol)/cm^{-1} 3532, 3456 (OH), 3300 (NH), 1692 (acid carbonyl), 1614 (C=C, benzene), 1528 and 1376 (conj. C-NO_2); δ_H (250 MHz, D_2O) 1.16-1.19 (6H, 2d, $CH\underline{Me}_2$), 1.63-1.89 (2H, m, 3-CH_2), 2.65-2.69 (2H, t, A_1 and A_2), 3.01 (2H, d, 4-CH_2), 3.26-3.39 (1H, septet, B), 3.63-3.72 (1H, m, 2-H), 6.87 (1H, s, 8-H) and 7.32 (1H, s, 5-H); EI m/z 293 (M^+), 205 (15%) and 72 (72). $C_{14}H_{19}N_3O_4$ requires M, 293.136.

3.9 6-(1-Phenylaminomethyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine arylamine (52)

Aniline (0.692g, 7.432mmol) in benzene (1ml) was added dropwise to a stirred solution of the aldehyde (**24**) (0.506g,1.827mmol) in benzene (50ml). The resultant solution was refluxed overnight with azeotropic removal of water, using Dean-Stark apparatus, cooled and evaporated to yield the crude anil (**51**) (1.505g). To a solution of the crude anil  in methanol (40ml), at 40°C, was added sodium borohydride (0.160g, 4.229mmol) and the resulting mixture refluxed for 1 hour. Water was added and the suspension extracted with diethyl ether. The ether extracts were washed with water, dried (anhydrous magnesium sulphate) and evaporated. Column chromatography on silica gel with chloroform, n-hexane, ethanol (1:1:1) as the eluent yielded the crude arylamine (**52**) (0.526g). Further purification by preparative TLC on silca gel with ethyl acetate:ethanol (80:20) as the mobile phase gave the amine (**52**) as a red oil (0.402g, 62%). This oil (**52**) proved difficult to recrystallize but solidified to a low melting solid. m.p 83.4-85.7°C; ν_{max} (film)/cm^{-1} 3392 (NH), 1616 and 1602 (C=C, benzene), 1524

and 1336 (conj. C-NO_2), 750 and 694 (aryl-H); δ_H (250MHz, $CDCl_3$) 1.18-1.22 (6H, 2d, $CH\underline{Me}_2$), 1.58 (1H, m, 3-H), 1.85 (1H, m, 3-H), 2.54-2.62 (3H, m, A_1, A_2, B), 2.90-2.96 (2H, dd, 4-CH_2), 3.49 (1H, m, 2-H), 4.3 (1H, br s, 1-H), 4.48 (2H, s, 9-CH_2), 5.30 (1H, br s, NH), 6.60 (2H, d, J8.1, Ar-H), 6.67-6.73 (1H, t, J8.1 and 6.1, Ar-H), 7.05 (1H, s, 5-H), 7.12-7.18 (2H, t, J8.1 and 6.1, Ar-H) and 7.23 (1H, s, 8-H); m/z EI 354.2033 (M^+, 15.6%), 93 (100) and 72 (80). $C_{20}H_{26}N_4O_2$ requires M, 354.2056.

3.10 6-Ethenyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetra-hydroquinoline, Oxamniquine olefin (38)

The reaction was performed in dry glassware under a continuous flow of nitrogen. To a suspension of triphenylphosphonium bromide (3.508g, 9.82mmol) in dry diethyl ether (50ml) was added n-butyl lithium (4.4ml, 9.836mmol). The resultant yellow suspension was stirred at room temperature for 4 hours. The aldehyde (**24**) (0.680g, 2.452mmol) dissolved in diethyl ether was added to the stirred suspension. The mixture was refluxed overnight, cooled, diluted with ether (100ml) and filtered through celite. The filtrate was washed with water, dried (calcium chloride) and evaporated to a red oil. Column chromatography on silica gel with chloroform-hexane-ethanol (1:1:1) as the eluent yielded the olefin (**38**) (0.428g, 63%) as a red viscous residue. Attempts to recrystallize the olefin were unsuccessful but the oil solidified, on cooling, to a low melting solid. ν_{max} (film)/cm^{-1} 3390 (NH), 3084 (olefinic C-H), 1618 and 1564 (C=C), 1522 and 1342 (conj. nitro); δ_H (250 MHz, $CDCl_3$) 1.08-1.12 (6H, 2d, $CH\underline{Me}_2$), 1.48-1.64 (2H, br m, 3-H, N^2-H), 1.91-2.00 (1H, septet, 3-H), 2.43-2.52 (1H, dd, A_2), 2.77-2.64 (3H, m, 4-CH_2, B), 2.67-2.93 (1H, dd, A_1), 3.27-3.36 (1H, m, 2-CH), 5.14 (1H, br s, 1-H), 5.19-5.24 (1H, dd, J11 and 1, *cis*10-H), 5.49-5.57 (1H, dd, J17.5 and 1, *trans*10-H), 7.05 (1H, s, 8-H), 7.00-7.12 (1H, dd, J11 and 17.5, 9-H) and 7.18 (1H, s, 5-H); m/z EI 275.1652 (M^+, 15.6%), 187 (94) and 72 (100). $C_{15}H_{21}N_3O_2$ requires M, 275.1634.

3.11 Attempted oxymercuration demercuration reaction of oxamniquine olefin (38)

Water (4 ml) was added to mercuric acetate (0.141g, 0.442mmol) and stirred to yield a colourless solution. THF (4 ml) was added to the mercuric acetate solution resulting in the formation of a yellow precipitate. A solution of the olefin (**38**) (120mg, 0.436mmol) in THF (12 ml) was added to suspension and stirred overnight at room temperature. To the yellow grey suspension obtained was added 3M sodium hydroxide (1.0 ml) and stirred to give a deep red coloration.

0.5M sodium borohydride dissolved in 3M sodium hydroxide (0.5 ml) was added and stirred. A grey precipitate was deposited. The suspension was stirred to allow coagulation of the mercury, the dark red aqueous THF supernate decanted off, saturated with potassium carbonate and extracted into diethyl ether. The ether extracts were washed with water, dried (magnesium sulfate) and evaporated *in vacuo* to a red residue (0.136g). TLC on silica gel with chloroform:ethanol:heptane (10:10:10) and n-butanol:acetic acid:water (4:1:5) showed a single spot with R_f value identical to that of the olefin (**38**). ν_{max} (film)/cm^{-1} 3380 (NH), 1616, 1564 (C=C), 1516 and 1344 (conj. C-NO_2); δ_H (60MHz, $CDCl_3$) 5.1-5.68 (2H, 2dd, 10-CH_2) and 6.85-7.38 (3H, m, 8-H, 9-H, 5-H).

3.12 6-(1-methyl-1-hydroxymethyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine secondary alcohol homologue (29)

The reaction was performed in dry glassware under a steady flow of nitrogen. An ethereal solution of methyl lithium (6.6ml, 9.2mmol) was added slowly to a stirred dichloromethane solution of titanium tetrachloride (9.1ml, 9.1mmol) cooled to -78°C. A purple-black adduct formed immediately and was allowed to warm to -20°C. A solution of the aldehyde (**24**) (0.510g, 1.841mmol) in anhydrous diethyl ether (30ml) was added to the vigorously stirred solution of the adduct at -20°C. The mixture was stirred at -20°C for 2h, poured into cold water (100ml), basified (2M NaOH) and extracted with three volumes of diethyl ether. The combined ethereal extract was washed with three volumes of water, dried (magnesium sulphate) and evaporated to dryness at the pump. Column chromatography on silica gel using chloroform-ethanol (70:30) as eluent followed by preparative TLC on silica gel using chloroform:ethanol (70:30) containing 1ml conc. ammonia as the mobile phase yielded a yellow solid. Recrystallization from ethyl acetate:ethanol gave the alcohol (**29**) (0.201g, 37%), m.p. 152.0-153.5°C. ν_{max} (nujol)/cm^{-1} 3304 (NH), 1618 (C=C), 1520 and 1330 (conj. C-NO_2); δ_H (250 MHz, $CDCl_3$) 1.06-1.10 (6H, dd, CH$\underline{Me}_2$), 1.512-1.64 (3H, br m 3-H, 9-OH, N^2-H), 1.53 (3H, d, J6.4, 10-Me), 1.98-1.99 (1H, m, 3-H), 2.42-2.50 (1H, dd, A_2), 2.72-2.86 (3H, m, 4-CH_2, B), 2.89-2.90 (1H, D, A_1), 3.31 (1H, m, 2-H), 4.98 (1H, s, 1-H), 5.20-5.28 (1H, q, J6.4, 9-H), 7.02 (1H, s, 5-H) and 7.29 (1H, s, 8-H); m/z EI 293.1730 (M^+, 9.5%), 205 (68) and 72 (100%). $C_{15}H_{23}N_3O_3$ requires M, 293.1739.

3.13 Attempted synthesis of 6-(oxiran-2-yl)-2-N-isopropylamino-methyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine epoxide (34)

3.13.1 Attempted alkaline hydrogen peroxide oxidation of 6-ethenyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4,-tetrahydroquinoline (38)

To a stirred, methanolic solution of the olefin (**38**) (0.137g, 0.498mmol) contained in a three-necked round bottomed flask and cooled in a water bath at 15°C was slowly added, from a dropping funnel, a methanolic solution of 14.8% hydrogen peroxide (0.17ml, 0.74mmol H_2O_2 in 10ml methanol). 2M sodium hydroxide (0.125ml) was the added and the reaction mix stirred at 15-17°C for one hour. The reaction mix was poured into ice-water (ca. 50ml) and the resulting suspension extracted with six volumes of diethyl ether. The ethereal extracts were washed with several volumes of water until the aqueous washings gave a negative test for the presence of peroxides with starch-iodide paper, dried (magnesium sulfate) and evaporated *in vacuo* to a viscous red film (0.113g). TLC of the reaction product on silica gel using chloroform:ethanol:heptane (10:10:10) and n-butanol:acetic acid:water (4:1:5) gave a single spot with an R_f value identical to that of the olefin (**38**). The IR and proton-NMR spectra were identical with those of the olefin (**38**). ν_{max} (film)/cm^{-1} 3392 (NH), 1618, 1564 (C=C), 1516 and1342 (conj. C-NO_2); δ_H (60MHz, $CDCl_3$) 5.25(1H, dd, J11 and 1, 10-H cis), 5.45 (1H, dd, J17.5 and 1, 10-H trans) and 6.85-7.40 (3H, dd overlaps 2s, 8-H, 5-H and 9-H).

3.13.2 Attempted epoxidation of 6-ethenyl-2-N-isopropylaminomethyl-7-nitro-tetrahydroquinoline (38) using urea hydrogen peroxide and acetic anhydride

The olefin (**38**) (0.157g, 0.571mmol), urea hydrogen peroxide (UHP) (0.464g, 4.936mmol) and disodium hydrogen phosphate (0.181g, 1.275mmol) were placed in a three-necked flask. Dichloromethane (ca. 15ml) was added and the mixture stirred to dissolve the olefin leaving the sodium phosphate suspended. The bright orange suspension was cooled in an ice bath and acetic anhydride (0.129g, 1.264mmol) dissolved in dichloromethane (1ml) added dropwise with stirring to the cold reaction mixture. The reaction mix was allowed to warm to room temperature and stirred for 4 hours when additional spots were observed on TLC. Water (10ml) was added to the reaction mix to dissolve the suspended inorganic salts. The organic phase was separated, washed with several volumes of water, dried (magnesium sulfate) and evaporated *in vacuo* to a viscous red residue(0.178g). TLC on silica gel, using chloroform:ethanol:heptane (10:10:10) as the mobile phase, revealed a mixture of products. ν_{max} (film)/cm^{-1} 3360 (NH), 3088 (olefinic CH), 1754 (carbonyl), 1616 (C=C), 1520 and 1344 (conj. C-NO_2);

δ_H (60 MHz, $CDCl_3$) 1.1 (6H, d, $CH\underline{Me}_2$), 2.15 (3H, s, $\underline{Me}CO$), 5.08-5.65 (2H, 2dd, 10-$C\underline{H}_2$) and 6.8-7.28 (3H, m, 8-H, 9-H, 5-H).

3.13.3 Reaction of 6-methanoyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline (24) with dimethylsulfonium methylide using a phase transfer system

Trimethylsulfonium iodide (0.155g, 0.759mmol), crushed potassium hydroxide pellets (0.4g) and the aldehyde (**24**) (0.205g, 0.740mmol) were placed in a 100 ml round bottomed flask and mixed. Water (0.04ml) and acetonitrile (30ml) were added and the mixture stirred vigorously at 60°C for 1 hour. The initially formed orange coloured heterogeneous system darkened to a burgundy-red suspension. The suspension was cooled to room temperature, filtered and the filter residue washed with diethyl ether. The combined organic extracts were evaporated to dryness. The residue obtained was triturated with diethyl ether, the yellow ether solution obtained washed with 2 volumes of water, dried (magnesium sulfate) and evaporated in vacuo to a yellow film (87mg). TLC of the crude product on silica gel, using chloroform:ethanol (70:30) as the mobile phase, showed a mixture of at least three components which proved difficult to isolate and characterize.

3.13.4 The Corey-Chaykvosky reaction of 6-methanoyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline (24) with dimethylsulfonium methylide

The reaction was performed in dry glassware under a continuous flow of nitrogen. The dimsyl ion was prepared from sodium hydride (0.023g, 0.95mmol) and DMSO (1.2ml) by heating at 70-75°C under nitrogen according to the method of Corey and Chaykovsky. The sodium hydride (60% oil dispersion) having first been washed 3 times with dry petroleum-ether (b.p.40-60) and flushed dry with nitrogen.The dimsyl ion was cooled to room temperature, diluted with dry THF (1.2ml), the reaction protected from light by wrapping with aluminium foil and cooled to -5°C using an ice-salt bath. To the stirred mixture was added a solution of trimethylsulfonium iodide (0.180g, 0.882mmol) dissolved in DMSO (1.0ml) over 3 minutes. The mixture was stirred for 1 minute longer then a solution of the aldehyde (**24**) (0.198g, 0.715mmol) in THF (5ml) was added over 5 minutes. Stirring was continued at -5°C for 15 minutes and then with the bath removed for 45 minutes. The reaction mixture was diluted with water (ca. 15ml) and extracted with 4 volumes of ethyl acetate. The combined extracts washed with 3 volumes of water, dried (anhydrous potassium carbonate) and evaporated to give a red film (0.203g).

Chromatographic separation of the crude on preparative silica gel TLC plates using chloroform:ethanol (70:30) containing 1ml conc. ammonia yielded two isolable compounds. A third component was unstable and could not be characterized. The non polar component, a deep red film, (26mg, 11%) was identified as the methylthio vinyl derivative (**67**). ν_{max} ($CHCl_3$)/cm^{-1} 3392 (NH), 1616 (s, C=C), 1508 and 1344 (conj C-NO_2); δ_H (250 MHz, $CDCl_3$) 1.09-1.13 (6H, 2d, CH<u>Me</u>$_2$), 1.56-1.60 (1H,m, 3-H), 1.92-1.98 (1H, m 3-H), 2.38 (3H, SMe), 2.46-2.55 (1H, q, A_2), 2.75-2.93 (4H, m, 4-CH_2, B, A_1), 3.34 (1H, m, 2-H), 5.19 (1H, s, 1-NH), 6.58 (1H, d, J17, *trans*H), 6.72 (1H, d, J 17, *trans* H), 7.06 (1H, s, 8-H), and 7.07 (1H, s, 5-H); m/z CI 322.1589 (M+H). $C_{16}H_{23}N_3O_2S$ requires M, 321.1511.

The second component, a yellow film (31mg, 14%), was identified as the diol (**31**); ν_{max} ($CHCl_3$)/cm^{-1} 3560 (OH), 3384 (NH), 1620 (C=C), 1510 and 1342 (conj C-NO_2), and 1070 (C-O); δ_H (250 MHz, D_2O) 1.06-1.10 (6H, 2d, CH<u>Me</u>$_2$), 1.51-1.59 (1H, m, 3-H), 1.91-1.94 (1H, m, 3-H), 2.42-2.52 (1H, m, A_2), 2.77-2.86 (4H, m, 4-CH_2, B, A_1), 3.25-3.29-(1H, m, 2-H), 3.54-3.63 (1H, m, J 8.5, 10-H), 3.86-3.93 (1H, m, J 8.5, 10-H), 5.17-5.22 (1H, m, 9-H), 7.04 (1H, s, 5-H), and 7.31 (1H, s, 8-H) ; m/z 310.1767 (M+H). $C_{15}H_{24}N_3O_4$ requires M, 309.1688.

3.14 Attempted preparation of 6-(oxiran-2-yl)-1-N-acetyl-2-(N-acetyl-N-isopropylaminomethyl)-7-nitro-1,2,3,4-tetrahydroquinoline (78)

The dimsyl ion was prepared from sodium hydride (0.022g, 0.925mmol) and DMSO (1.2ml) by heating at 70-75°C under nitrogen according to the method of Corey and Chaykovsky. The dimsyl ion was cooled to room temperature and diluted with dry THF (1.2ml). The reaction vessel was protected from light by wrapping with aluminium foil and cooled to -5°C using an ice-salt bath. To the stirred mixture was added a solution of trimethylsulfonium iodide (0.140g, 0.686mmol) dissolved in DMSO (1.0ml) over 3 minutes. The mixture was stirred for 1 minute longer then a solution of the diacetyl derivative (**48**) of oxamniquine aldehyde (0.204g, 0.565mmol) dissolved in THF (10ml) was added over 2 minutes. The reaction mix was stirred at -5°C for 15 minutes and then at room temperature for 60 minutes. The reaction mix was diluted with water (30 ml) and extracted with three volumes of ethyl acetate. The combined extracts were washed with three volumes of water, dried (anhydrous potassium carbonate) and evaporated *in vacuo*. A yellow glass was obtained (0.177g). TLC revealed the presence of three components. Column chromatography on silica gel using

gradient elution with ethyl acetate:ethanol mixtures yielded two products. The major product, isolated as a yellow powder (62mg, 34%) was identified as N^2-acetyl oxamniquine aldehyde (**49**). ν_{max} ($CHCl_3$)/cm-1 3314 (NH), 1672 (aryl aldehyde), 1624 (amide), 1604 (C=C), 1528 and 1344 (conj. C-NO_2); δ_H (250 MHz, $CDCl_3$) 1.19-1.27 (6H, 2d, CH<u>Me</u>$_2$), 1.59-1.72 (1H, m, 3-H), 1.93-1.99 (1H, m, 3-H), 2.22 (3H, s, N^2-CO<u>Me</u>), 2.81-2.87 (2H, m, 4-CH_2), 3.05-3.11 (1H, dd, A_2), 3.42-3.50 (1H, m, A_1), 3.61-3.69 (1H, dd, 2-H), 4.03-4.11 (1H, m, B), 6.98 (2H, s, 8-H, 1-H), 7.61 (1H, s, 5-H) and 10.11 (1H, s, 9-H); m/z 319 (M^+, 15%), 205 (65), 115 (88) and 72 (100). (Found: C, 59.8; H, 6.7; N, 12.9%; M^+, 319.1514. $C_{16}H_{21}N_3O_4$ requires C, 60.2; H, 6.6, N, 13.2%, M, 319.1532).

The unreacted diacetyl derivative (**48**) was also recovered (60mg, 29%). A third component proved difficult to isolate and characterize.

3.15 6-Ethanoyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetra-hydroquinoline, Oxamniquine 6 methyl ketone (37)

To a volume of refluxing toluene (ca. 250ml) was added the alcohol (**29**) (0.154g, 0.526mmol) dissolved in toluene (5ml) followed by cyclohexanone (4ml). After 3 hours reflux, during which time toluene distillate was collected, aluminium isopropoxide (0.06g, 0.294mmol) dissolved in toluene (5ml) was added over 10 minutes. The mixture was refluxed for a further 6 hours, until only a small volume remained, quenched with water, cooled to room temperature and extracted with 2M HCl and two volumes of water. The aqueous extracts were basified with 2M NaOH and back extracted into dichloromethane. The organic extracts were washed with several volumes of water, dried (magnesium sulfate) and evaporated. Column chromatography on silica gel with chloroform:ethanol (95:5) as the eluent yielded the ketone (**37**) (0.104g, 68%), m.p. 222.9°C (decomp) (from diethyl ether). ν_{max} ($CHCl_3$)/cm^{-1} 3384 (NH), 1674 (aryl C=O), 1608 (C=C), 1516 and 1330 (conj C-NO_2) ; δ_H (250 MHz, $CDCl_3$) 1.09-1.13 (6H, 2d, CH<u>Me</u>$_2$), 1.48-1.57 (1H, m, 3-H), 1.78 (1H,br s, N<u>H</u>CH), 1.95-2.02 (1H, m 3-H), 2.46 (3H, s, 10-Me), 2.42-2.51 (1H, t, A_2), 2.77-2.89 (4H, m, 4-CH_2, B), 2.92-2.98 (1H, dd, A_1), 3.29-3.41 (1H, m, 2-H), 5.64 (1H, s, 1-H), 6.81 (1H, s, 8-H) and 7.19 (1H, s, 5-H) ; m/z EI 291.1573 (M^+), CI 292 (M+H,100%) and 262 (40) and 72 (20). $C_{15}H_{21}N_3O_3$ requires M, 291.1583.

3.16 Attempted synthesis of 6-(2-methyloxiran-2-yl)-2-N-isopropyl-aminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline (82)

The reaction was conducted in dry glassware under a steady stream of nitrogen. The dimsyl ion was prepared from sodium hydride (15mg, 0.625mmol) and DMSO (1.2ml) by heating at 70-75°C as previously described. After cooling to room temperature the dimsyl ion was diluted with THF (1.2ml) and cooled to -5°C. A solution of dimethylsulfonium iodide (76 mg, 0.275mmol) dissolved in DMSO (1.0ml) was added with stirring. After 1 minute the ketone (**37**), (80mg, 0.275mmol) dissolved in THF (5ml) was added over 3 minutes with stirring. The mixture was stirred at -5°C for 1 hour and then at room temperature for an additional hour. The reaction mix was diluted with water (15ml) and extracted with three volumes of ethyl acetate. The extracts were washed with three volumes of water, dried (anhydrous potassium carbonate) and evaporated *in vacuo* to a brown film (70mg). TLC on silica gel, using chloroform:ethanol (70:30) containing 1ml conc. ammonia as the mobile phase, showed the presence of a streaking spot with R_f value similar to that of the ketone (**37**). ν_{max} ($CHCl_3$)/cm^{-1} 3395 (NH), 1675 (aryl C=O), 1610 (C=C), 1525 and 1348 (conj. C-NO_2); δ_H (250MHz, $CDCl_3$) 1.06-1.10 (6H, 2d, CH$\underline{Me_2}$), 1.49-1.52 (1H, m, 3-H), 1.63 (1H, br s, N^2-H), 1.95-1.96 (1H, m, 3-H), 2.42 (3H, S, 10-CH_3), 2.41-2.50 (1H, m, A_2), 2.77-2.50 (4H. m. 4-CH_2, A_2, B), 3.30-3.34 (1H, m, 2-H), 5.61 (1H, S, 1-H), 6.86 (1H, s, 8-H) and 7.20 (1H, S, 5-H).

3.17 6-(2-Methoxyethenyl)-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, Oxamniquine methyl vinyl ether (39)

The reaction was performed in dry glassware using dry solvents and reagents and under a steady stream of nitrogen. Lithium di-isopropylamide (LDA) was prepared from di-isopropylamine (0.7ml, 4.994mmol) and n-butyl lithium (2.8ml, 4.48mmol) at 0°C, under nitrogen. Diphenylmethoxymethyl phosphine oxide (0.902g, 3.663mmol) in dry THF (50ml) was added, over 15 minutes, to the stirred LDA at 0°C. The resultant orange solution was stirred and cooled to -78°C. After 0.5 hours the aldehyde (**24**) (0.509g, 1.838mmol) dissolved in THF (20ml) was added, the mixture allowed to warm to room temperature and stirred overnight under nitrogen. The reaction mixture was poured into saturated ammonium chloride (100ml), basified with 2M NaOH and extracted with 3 volumes of diethyl ether. The combined ether extracts were washed with saturated sodium chloride, water, dried (magnesium sulfate) and evaporated *in vacuo* to a burgundy-red residue (1.040g). This crude product was taken up in THF (50ml) and stirred with NaH (0.130g, 5.425mmol) for 20 hours at room

temperature protected from light. The resulting mixture was poured into water, basified with 2M NaOH and extracted with 3 volumes of diethyl ether. The ether extracts were washed with saturated sodium chloride and water, dried (magnesium sulfate) and evaporated to a red residue (0.587g). Chromatography on silica gel with chloroform:ethanol (90:10) yielded the vinyl ether (**39**) (0.134g, 36%). ν_{max} ($CHCl_3$)/cm^{-1} 3396 (NH), 1616 (C=C), 1508, 1340 (conj C-NO_2), 1294 and 1096 (C-O); δ_H (250 MHz, $CDCl_3$), 1.10-1.14 (6H, 2d, CH<u>Me</u>$_2$), 1.52-1.68 (2H, br m, 3-H, N<u>H</u>CH), 1.90-1.91 (1H, m, 3-H), 2.47-2.55 (1H, t, A_2), 2.73-2.92 (4H, m, 4-CH_2, B, A_1), 3.34 (1H, m, 2-H), 3.68 and 3.73 (3H, 2s, trans and cis 10-O<u>Me</u>), 5.14 (1H, br s, 1-H), 5.59 (0.5H, d, J9 *cis* H), 6.09 (0.5H, d, J9, *cis* H), 6.31 (0.5, d, J13, *trans* H), 6.83 (0.5, d, J13, *trans* H), 6.96 (0.5H, s, Ar-H), 7.01 (0.5H, s, Ar-H), 7.11 (0.5H, s, Ar-H) and 7.60 (0.5H, s, Ar-H); m/z EI 305.1693 (M^+, 26.8%), 233 (80), 217 (100) and 72 (75). $C_{16}H_{23}N_3O_3$ requires 305.1739.

3.18 Attempted hydrolysis of 6-(2-methoxyethenyl)-2-N-isopropyl-aminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline (39)

The vinyl ether (**39**) (88mg, 0.289mmol) was taken up in THF (10 ml). To the stirred solution was added 30% $HClO_4$ (1 ml) resulting in darkening of the solution. The solution was stirred at room temperature for 48 hours, under nitrogen and protected from light. The reaction was quenched by pouring into water, basified with 2M NaOH to pH 12 and extracted with 3 volumes of dichloromethane. The organic extracts were washed with 3 volumes of water, dried (magnesium sulfate) and evaporated to a red film (60 mg). Preparative TLC on silica gel using chloroform:ethanol (70:30) containing 1ml conc. ammonia solution as the mobile phase yielded a brick red residue of the aldehyde (**24**) (28 mg, 35%). ν_{max} ($CHCl_3$)/cm^{-1} 3389 (NH), 1673 (aryl aldehyde), 1605 (C=C), 1522, 1345 and 1331 (aryl conj C-NO_2); δ_H (250 MHz, $CDCl_3$) 1.07-1.12 (6H, 2d, CH<u>Me</u>$_2$), 1.45-1.54 (1H, m, 3-H), 1.98-1.99 (1H, m, 3-H), 2.38-2.47 (1H, dd, A_2), 2.77-2.86 (3H, m, 4-CH_2 and B), 2.97 and 3.01 (1H, dd, A_1), 3.32-3.36 (1H, m, 2-CH), 5.87 (1H, br s, 1-H), 6.99 (1H, s, 8-H), 7.64 (1H, s, 5-H) and 10.12 (1H, s, 9-H); m/z EI 277.1431 (M^+). $C_{14}H_{19}N_3O_3$ requires M, 277.1426.

3.19 Attempted hydrolysis of 6-(2-methythioethenyl)-2-N-isopropyl-aminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, oxamniquine methylthio vinyl derivative (67)

3.19.1 Attempted hydrolysis with mercuric chloride

To a solution of the methylthio vinyl derivative (**67**) (12mg, 0.037mmol) dissolved in acetonitrile:water (3:1) (3ml) was added a solution of $HgCl_2$ (22mg, 0.081mmol) in the same solvent mixture. The resulting homogeneous solution was refluxed for 40 hours. The suspension obtained was filtered through celite, washed with one volume of saturated $NaHCO_3$, brine, dried (magnesium sulfate) and evaporated to a red film (14mg). The starting material, the methylthio vinyl derivative (**67**), was the major product recovered.

3.19.2 Attempted hydrolysis via the thioacetal

Hydrogen chloride-saturated benzene (10ml) was added to the methylthio vinyl derivative (**67**) (15mg, 0.047mmol), under nitrogen. Thiophenol (30mg, 0.272mmol) was added by syringe to the stirred suspension and the resulting mixture stirred at room temperature for 1.5 hours. The reaction mix was evaporated *in vacuo* to a red film (22mg). This product was treated with a solution of $HgCl_2$ (25mg, 0.094mmol) in a mixture of THF (4ml) and water (1ml). The reaction mix was stirred under nitrogen at room temperature overnight and the solvents removed *in vacuo*. The crude product was suspended in water, basified with 2M NaOH to pH 10 and extracted with 3 volumes of chloroform. The organic extracts were washed with water, dried (magnesuim sulfate) and evaporated to a red film (21mg). The unreacted methylthio vinyl derivative (**67**) was the major product recovered.

3.20 Attempted synthesis of 6-bromomethyl-2-N-isopropylamino-methyl-7-nitro-1,2,3,4-tetrahydroquinoline, oxamniquine bromide (35)

3.20.1 Reaction with carbon tetrabromide and triphenylphosphine

To a solution of oxamniquine (**1**) (98mg, 0.351mmol) in dry THF (15 ml), stirred under nitrogen, was added dry triphenylphosphine (105mg, 0.4mmol) followed by carbon tetrabromide (138mg, 0.416mmol) and stirred at room temperature for 1 hour. The resulting yellow suspension was diluted with ethanol to a clear solution and evaporated *under vacuo* to a yellow film (284 mg). Chromatographic separating on a silica column using chloroform:ethanol (50:50) as the eluent yielded a yellow solid (104 mg), m.p. 270°C (decomp). ν_{max} (nujol)/cm^{-1} 3557

(OH), 3306 (NH), 2403 (NH_2^+), 1626 (C=C), 1526 and 1331 (conj. C-NO_2) and 1058 (C-O stretch).

The product was dissolved in water, basified with 1M sodium hydroxide to pH 10, back extracted with chloroform, washed, dried and evaporated to a yellow solid. ν_{max} ($CHCl_3$)/cm^{-1} 3620 (OH), 3400 (NH), 1623 (C=C), 1525 and 1338 (conj. C-NO_2) and 1046 (C-O stretch).

3.20.2 Reaction with hydrobromic acid in acetic acid

A solution of oxamniquine (1) (115mg, 0.412mmol) dissolved in acetic acid (5 ml) was added to a stirred and cooled solution of 33% hydrobromic acid (10 ml) and stirred overnight at room temperature. Addition of diethyl ether (10 ml) to the suspension obtained resulted in the deposition of a white precipitate which was collected at the pump (169 mg). ν_{max} (nujol)/cm^{-1} 2340 (broad, NH_2^+), 1602 (C=C), 1523 and 1343 (conj. C-NO_2). The salt was suspended in triethylamine (100ml) and extracted with 3 volumes of chloroform, the extracts washed with water, dried (magnesuim sulfate) and evaporated to a yellow film. TLC on silica gel using chloroform:ethanol (70:30) showed several components.

CHAPTER FOUR

THE CENTRAL NERVOUS SYSTEM EFFECTS OF OXAMNIQUINE AND ANALOGUES

4.1 INTRODUCTION

The aims of this work were firstly to develop an *in vivo* model system capable of detecting convulsant activity and secondly to evaluate the effects of oxamniquine (1) and some of its analogues on the central nervous system (CNS).

4.1.1 Background and Rationale

(a) Oxamniquine side effects

Oxamniquine (1) (Vansil®) is a potent schistosomicide active principally against *S. mansoni* . This drug has been in general use since 1975 and about 9 million patients suffering from mansonian schistosomiasis have been treated in South America, the Caribbean area, Africa and the Middle East.[14] Although the drug is relatively well tolerated in clinical use, it induces certain adverse CNS effects. Several reports of seizures and/or electroencephalographic disturbances associated with the therapeutic use of oxamniquine in patients with schistosomiasis have been documented.[39,40,42] These incidences occurred in patients who have, and in some who had not had a previous history of epilepsy or other seizure disorder.

In a study of 180 Brazilian patients with *S. mansoni* infection treated with single oral doses of oxamniquine, the main neuropsychiatric side-effects seen were: drowsiness (50.6%), dizziness (41.1%), headache (16.1%), temporary amnesia (2.2%), behavioural disturbances (1.7%), chills (1.1%) and seizures (1.1%).[41] Oxamniquine induces convulsions in only a small proportion of the population and there are fourteen documented cases of convulsions after treatment in an estimated 9 million treatments.[14] Auditory or visual hallucinations, or both, have been recorded in closely monitored studies in Brazil, with incidences of 0.4 to 0.8%. However, since the drug is mainly used in rural areas and with considerable self-medication, the true incidence of these neuropsychiatric side effects in routine practice cannot be determined.[14]

In preclinical toxicological studies of oxamniquine in experimental animals, convulsions were reported to occur in some rodents dosed with the drug.[32] However, no details of the dose, the route of administration or the experimental protocol were given in this report. Other, generally nonspecific effects observed included hypomotility, malaise and some motor incoordination. Chronic administration of oxamniquine to beagle dogs, at doses of 20 or 30 mg/kg/day for five days every four weeks, was found to be associated with neurological disturbances of short duration, characterized by disturbance of gait, posture and

behaviour.[32] These disturbances tended to increase in severity from one dosage period to the next, and no organic lesion was demonstrated to correlate with these signs of neurological disorder.[32]

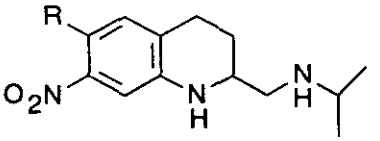
Oxamniquine has been shown to attain significant concentrations in the brain after intravenous administration of a 15 mg/kg dose to female Wistar rats.[18] This confirmed the prediction, from the hydrophobicity parameter (Log P) calculated from the experimentally determined octanol/water partition coefficient, that oxamniquine was capable of penetrating into the CNS.[19] The value for the nonionized species is 2.245, S.D. 0.064 and therefore in the range of those neutral compounds known to penetrate into the CNS in significant concentrations.[19] Peak brain concentrations of oxamniquine were obtained one hour after dosing and at all sampling times brain levels were higher than the corresponding plasma level. It seems logical that the central effects of oxamniquine will be related to high levels of the drug in the CNS.[18]

The major advantages of oxamniquine are its specificity of action against *S. mansoni* infection and its suitability for mass chemotherapy on an out-patient basis. However, the possible occurrence of severe CNS side effects has necessitated the administration of oxamniquine under close medical supervision especially in those with a known history of seizure disorders. In these cases a concomitant dose of an anticonvulsant has been advocated.[8] This has limited the usefulness of oxamniquine for mass chemotherapy and prompted the inclusion of a cautionary label in the monograph.[8,119]

A study was therefore undertaken to investigate whether any CNS effects of oxamniquine could be demonstrated in an *in vivo* model system. It was also the intention to study the CNS effects of the oxamniquine analogues synthesized in order to evaluate the effect of structural modification of the molecule on CNS activity. Of particular interest was the carboxylic acid metabolite (**9**) which is known to be present in high levels in the systemic circulation of subjects dosed with oxamniquine.

In studies of mice dosed orally with radio-labelled oxamniquine, 50mg/kg, it has been shown that at the time of peak serum levels of drug-related material (approximately 1.5 hour following a single dose) only about 10 % of the material, comprised of the unchanged oxamniquine, indicating the presence of high levels of metabolites in the systemic circulation.[25] It was anticipated that the study would give some indication of the contribution of this metabolite (**9**) to the central effects of oxamniquine.

It was also anticipated that the CNS activity of the novel alcohol (**29**) which is the secondary alcohol homologue of oxamniquine could be evaluated in the study.



R	
CH_2OH	(1)
CO_2H	(9)
$CH(OH)CH_3$	(29)

(b) Epilepsy

The term epilepsy is used collectively to include a group of syndromes of recurrent self-sustained paroxysmal disorders of brain function which are characterized by sudden, transientory, excessive discharge of cerebral neurones and involving one or more of the following systems: motor (convulsions), sensory, autonomic or psychic.[120] There may be excessive inhibition as well as excessive excitation, the one predominating over the other in different types of seizures as well as at different times in the course of a single seizure. The seizures are associated with loss or disturbance of consciousness and are nearly always accompanied by abnormal and excessive discharges in the electroencephalogram (EEG). The most prominent feature of an epileptic seizure is sustained synchronous neuronal discharges. Convulsive disorders and seizure disorders have been used as synonyms of the term epilepsy. Seizures however, refer to electroclinical events whereas epilepsy indicates a tendency for recurrent seizures.[121]

Epilepsy in man is characterized by chronically recurring, spontaneous clinical seizures of which there is a wide variety of clinical and electrical manifestations. This is due firstly, to the functional characteristics of the neuronal circuits primarily involved and their pattern of spread in the brain. Secondly it relates to the nature of the epileptic disorder itself, whether it is predominantly excitatory or inhibitory, whether of short or long duration and the degree of synchronization and quantity of neurones involved.

Various mechanisms of epilepsy have been proposed to account for the diversity of seizure types. It has been suggested that synchronous firing may require specifically timed inhibitory and excitatory neuronal activity. However, the specific mechanism for sustaining the synchronous firing is still unknown.

Physiological and biochemical factors that may facilitate the spread of abnormal electrical activity to other parts of the brain include: changes in blood gas tension, blood glucose levels, plasma pH and electrolyte composition of extracellular fluid, fatigue, sleep deprivation, drug withdrawal, nutritional deficiencies, emotional stress and endocrine changes. Alterations in the membrane or metabolic properties of individual neurons may render the neurons pathologically hyperexcitable. Such changes may in turn affect Na^+ and Ca^{2+} conductances.

Biochemical lesions affecting the synthesis, storage, release and reuptake of inhibitory amino acid neurotransmitters may be another possible mechanism for enhancing neuronal excitability. Many anticonvulsants are known to affect the metabolism of γ-aminobutyric acid (GABA), a major inhibitory neurotransmitter in the CNS. Some of the antiepileptic properties of the benzodiazepines maybe related to the receptor complex involving both benzodiazepine and GABA receptors that are linked intimately to chloride conductance and synaptic inhibition.

The classification of epileptic seizures based on clinical manifestations and EEG as adopted by the International League Against Epilepsy (ILAE) is presented in Table 4.1.[121]

Table 4.1 International classification of epileptic seizures[121]

I. Partial (focal, local) seizures
- A. Simple partial seizures
- B. Complex partial seizures
  1. With impairment of consciousness at onset.
  2. Simple partial onset followed by impairment of consciousness
- C. Partial seizures leading to generalized tonic-clonic seizures (GTC)
  1. Simple evolving to GTC
  2. Complex evolving to GTC (including those with simple partial onset)

II. Generalized seizures (convulsive or nonconvulsive)
- A. 1. Absence - brief attack with sudden onset and termination, impairment of consciousness.
  2. Atypical absence - slower onset and cessation than those with absence seizures.
- B. Myoclonic - brief single, mild to moderate jerks of arms or head.
- C. Clonic - rhythmic, multiple jerks of all parts of the body with loss of consciousness.
- D. Tonic - rigid, violent muscular contractions, fixing limbs in some strained position. loss of consciousness.
- E. Tonic-clonic - loss of consciousness associated with generalized tonic muscle contraction followed by rhythmic contractions of the limbs.
- F. Atonic - sudden loss of muscle tone, leading to a head drop or slumping to the ground.

III. Unclassified epileptic seizures (includes some neonatal seizures)

(c) The management of epilepsy

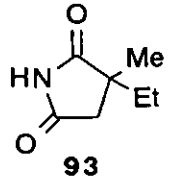
The management of epilepsy involves the use of antiepileptic drugs. An antiepileptic drug may be defined as a drug which when administered over a prolonged period of time will decrease the incidence or severity of spontaneous seizures occurring in patients with epilepsy.[122] Antiepileptic drugs suppress but do not cure epilepsy. The main objective of using antiepileptic drugs is therefore to suppress seizures and seizure recurrence. Unfortunately the currently available drugs do not suppress seizure activity in all epileptics. The therapeutic success appears to be dependent on the type of seizure and the accompanying neurological abnormalities. The clinical classification of some antiepileptic drugs is as shown in Table 4.2.[122]

Table 4.2 Clinical classification of some epileptic drugs.[122]

Drug	Generalized tonic-clonic and partial seizures	Myoclonic seizures	Generalized absence seizures
Phenytoin (**96**)	++	-	-
Carbamazepine (**97**)	++	-	-
Sodium valproate (**98**)	++	++	++
Phenobarbital (**95**)	+	+	-
Clonazepam (**94**)	+	++	+
Ethosuximide (**93**)	-	-	++

++, efficacy against the seizure type at non-toxic serum concentrations.
+, efficacy against the seizure type at toxic serum concentrations.
-, no efficacy

There is clear differentiation between drugs that are effective only in absence-type seizures [*e.g.* ethosuximide (**93**)], those that are effective in myoclonic seizures [benzodiazepines *e.g.* clonazepam (**94**), barbiturates *e.g.* phenobarbitone (**95**)], those effective in focal and secondarily generalized seizures [phenytoin (**96**)], carbamazepine (**97**)], and those active in all these seizures [sodium valproate (**98**)].



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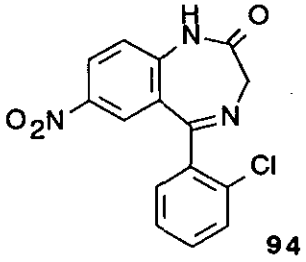

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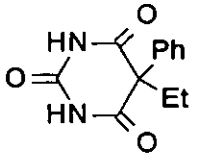

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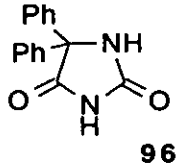

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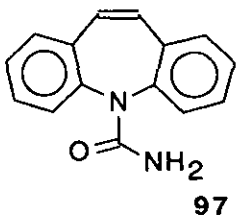

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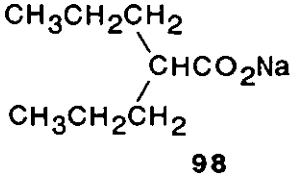

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The actions of antiepileptic drugs on receptor sites and their associated ion channels provide the most direct and specific mechanism for modifying inhibitory and excitatory transmission. Significant effects on seizures can be produced by drugs that either modify the synthesis or further metabolism of the neurotransmitters or alter their release or uptake. Enhancement of inhibition is therefore the most effective means of decreasing abnormal excitability. The most powerful and general effects involve compounds acting on GABA-mediated inhibition. Effects on excitatory transmission mediated by glutamate or aspartate or related compounds are also potentially important.

(e) Experimental models of epilepsy

Since CNS convulsants have no therapeutic indication, animal models designed to demonstrate convulsant activity have been developed primarily to screen potential anticonvulsant drugs for use in the treatment of epilepsy. The convulsant activity of a drug compound is normally encountered as a side effect in routine screening of the drug in animal models for pharmacological activity and is generally not tested for specifically.

The development of experimental models of epilepsy has been necessitated by the search for new antiepileptic drugs and the need to detect anticonvulsant activity. It has been demonstrated that drugs effective in epilepsy can be identified by testing their ability to suppress experimentally induced convulsions in normal laboratory animals. Since then, different species of laboratory animals have been subjected to a variety of electrical, chemical and sensory-evoking techniques in anticipation of finding a model that would be more representative of one or more of the types of human epilepsy.[123]

The ideal model of epilepsy is one that is representative of the clinical disorder. However, knowledge of the underlying causes of various types of convulsive disorders is still incomplete and the development of laboratory models based on etiology is not yet possible.[124]

Drug screening for potential antiepileptic activity has two primary objectives; firstly, to discover compounds capable of elevating the seizure threshold and secondly, to identify compounds that prevent the spread of seizure discharge through neural tissue within the brain and central nervous system, protecting against minimal threshold seizure and maximal seizures respectively. Virtually all screening tests depend on suppression of chemically or electrically induced seizures by the single-dose administration of the test drug and are traditionally

carried out in rodents. The five tests used for routine identification, quantification and evaluation of anticonvulsant activity are: (i) maximal electro-shock seizure test, (ii) subcutaneous pentylenetetrazole seizure threshold test, (iii) subcutaneous bicuculline seizure threshold test, (iv) subcutaneous picrotoxin seizure threshold test and (v) subcutaneous strychnine seizure pattern test.[124] Special tests performed to differentiate possible mechanisms of action include GABA receptor binding studies and adenosine uptake studies. The tests are chosen firstly, for their ability to detect substances with anticonvulsant activity, secondly, to determine whether such activity results from prevention of seizure spread or from elevation of seizure threshold and lastly to provide some insight into their mechanisms of action.[124]

Three distinct actions of anticonvulsant drugs on neuronal processes can be cited as underlying the effects of anticonvulsants in experimental seizures no matter how the seizures are induced.[125] These are stabilization of the neuronal membrane, decrease in tendency to repetitive discharge, and a reduction in spread of seizure discharge. Elevation of threshold for seizure discharge and reduction of spread of seizure discharge are the two principle effects of anticonvulsants that are seen in all models of epilepsy. The anticonvulsant drugs can induce these effects either by increasing the degree of inhibition or by blocking excitation in the brain processes that involve action at the synapses.

The intensity of the convulsive stimulus is of major importance in evaluating the anticonvulsant properties of chemical substances. It has been demonstrated that the specificity of experimental models of epilepsy is primarily due to the intensity rather than the nature of the stimulus used or the kind of seizure component evoked.[124] Electrically or chemically-induced, generalized seizure models are the most commonly used models for the assay of anticonvulsant drugs.

The maximal electro-shock (MES) test, used for the determination of activity against maximal electroconvulsive shock, is used to identify compounds that prevent seizure spread.[123] The ability of a compound to prevent maximal electro-shock seizures is believed to be correlated with its ability to prevent the spread of seizure discharge through neural tissue and is thought to indicate potential efficacy in the treatment of *grand mal* seizures. Phenytoin is the antiepileptic drug best known for its selective action in preventing maximal seizures.

The ability of a compound to prevent threshold seizures induced by subcutaneous pentylenetetrazol, the Metrazole test, has been correlated with the ability to raise the threshold for excitation of neural tissue.[123] Selective action in this test is

believed to indicate efficacy against absence (*petit mal*) seizures. The benzodiazepines are the most potent drugs to selectively inhibit pentylenetetrazole-induced threshold seizures.

The subcutaneous strychnine, picrotoxin and bicuculine tests are used to differentiate the mechanisms by which the candidate substances exert their anticonvulsant activity.[126]

(e) Systemic Chemical Convulsants

Convulsants or analeptics are those drugs producing CNS stimulation as their principle action. Several other classes of drugs cause CNS stimulation as a side effect of their principal therapeutic effect. All convulsants are capable of generalized CNS stimulation leading to generalized convulsions. Analeptics have previously been used to counteract the effects of CNS depressants, especially respiratory depression. However, their safety for selective respiratory centre stimulation is narrow and unpredictable and therefore they have little or no therapeutic value.

Normal functioning of the CNS is dependent on a balance between excitatory and inhibitory processes in the brain which are normally maintained within narrow limits. The principle neurotransmitters in the CNS mediating excitatory responses are glutamic acid (**99**) and aspartic acid (**100**). γ-Gamma amino butyric acid (GABA) (**101**) and glycine (**102**) are the main inhibitory neurotransmitters. Drugs may increase CNS excitability by one of two methods; blocking inhibition or enhancing excitation. A reduction in inhibition as is induced by strychnine or an enhancement of excitation as induced by pentylenetetrazole alter the delicate balance towards seizure activity. In human epilepsy, the normal CNS balance is upset resulting in seizure activity.

$HO_2CCH_2CH_2CH(NH_2)CO_2H$ **(99)**

$HO_2CCH_2CH(NH_2)CO_2H$ **(100)**

$HO_2CCH_2CH_2CH_2NH_2$ **(101)**

$HO_2CCH_2NH_2$ **(102)**

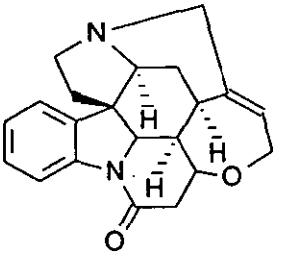
The convulsant agents strychnine (**103**), pentylenetetrazole (**104**) and picrotoxin are known to induce epileptiform cortical electrographic patterns. These drugs are commonly employed as systemic chemical convulsants in producing experimental models of epilepsy for the screening or investigation of anticonvulsant drugs and in the study of CNS neurotransmitters and CNS receptor types.

(i) Strychnine

Strychnine is an indole alkaloid obtained from the seeds of *Strychnos nux-vomica L.* and the seeds of other species of *Strychnos*. Strychnine causes excitation of all parts of the CNS, increasing the level of neuronal excitability by selectively blocking inhibition, resulting in the enhancement of on-going neuronal activity and the exaggeration of reflex effects produced by sensory stimuli. Strychnine is a powerful convulsant and produces a characteristic motor pattern which in laboratory animals is typified by tonic extension of the body and all limbs and spinal convulsions.

The mechanism of action of strychnine is due to interference of post-synaptic inhibition mediated by glycine. Glycine is an inhibitory neurotransmitter to receptor sites in the CNS. Strychnine selectively and competitively antagonizes the inhibitory effects of glycine at all glycine post-synaptic receptor sites. Although all levels of the CNS are affected, the actions on the spinal cord predominate. Here strychnine blocks the binding of glycine to motor neurones leading to uncontrolled excitation of skeletal muscles resulting in tetanic convulsions. Fatal strychnine poisoning results from central respiratory depression.

Strychnine is highly toxic and although formerly used as a bitter and analeptic, it now has no therapeutic use. It is, however, used under strict control as a rodenticide and in experimental pharmacology for the screening of anticonvulsant drugs for use in the treatment of epilepsy.



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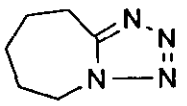
(ii) Pentylenetetrazole

Pentylenetetrazole (Metrazole®, Leptazole®) **(104)** is the most commonly used systemic chemical convulsant for the induction of epileptiform seizures in experimental models of epilepsy.[125] At high enough doses this agent affects all parts of the central nervous system, but the most sensitive region is the cerebral cortex. The major action of pentylenetetrazole is to reduce GABAimergic inhibition resulting in increased CNS excitability. Direct excitatory effects may

also be involved and the drug has previously been referred to as a synaptically acting convulsant with the implication that it directly activates excitatory synapses. However, the activating mechanism remains unexplained. Pentylenetetrazole does not directly activate muscle or nerve fibres. It has also been suggested that the mechanism of action might be to potentiate the response to a stimulus rather than to initiate activity.



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(iii) Picrotoxin

Picrotoxin is a non-nitrogenous neutral compound isolated from the seeds of *Amarita cocculus*. Picrotoxin is a powerful stimulant which affects all parts of the CNS, inducing clonic uncoordinated convulsions accompanied by salivation, increased blood pressure, vasomotor and respiratory stimulation. The effects of picrotoxin are similar to those of pentylenetetrazole except that they develop more slowly after injection. Muscle twitching is not noticeable at first but appears early in the build up and gradually increases, merging into a severe clonic convulsion. Picrotoxin selectively antagonizes the inhibitory effects of GABA at all levels in the CNS. It is thought to interact with sites closely associated with the ionophores that are coupled with GABA receptors which permit chloride influx.[127]

Other systemic convulsants commonly used as laboratory tools for the induction of epileptiform seizures in the investigation or screening of anticonvulsant drugs include bemegride and bicuculine.

(iv) Bemegride

Bemegride causes general stimulation of the CNS and its actions are comparable to those of pentylenetetrazole. This drug is used clinically as an analeptic and as an activator in the diagnosis of focal epilepsy. The effectiveness of bemegride in the diagnosis of focal epilepsy suggests usefulness in detecting convulsive activity in other agents that is it should potentiate them.

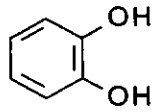
(v) Bicuculine

Bicuculine is a convulsant alkaloid from *fumaraceous* plants. Intravenous administration of bicuculine immediately induces epileptiform seizures. Baboons, given adequate doses, develop a status epilepticus in which seizures may be sustained for 150 to 300 minutes without interruption by isoelectric periods.

Bicuculine is a $GABA_A$ receptor antagonist and its mode of action is believed to be via blockade of inhibitory systems employing GABA as the inhibitory neurotransmitter.

(vi) Catechol

Catechol (**105**) has been shown to induce dose dependent increase in motor activity which may be seen even in surgically anaesthetized animals.[128,129] This effect is characterised by increased breathing rate, increased heart rate, muscle twitches and spontaneous convulsions. The mechanism involved is central and the effects resemble human epilepsy in that they may be suppressed by the administration of antiepileptic drugs. Low doses of catechol also produce a hyperexcitable state in which muscular jerks or spontaneous convulsions may be induced by sensory stimulation. Catechol has a short duration of action due to its rapid metabolism and renal excretion. The duration of action is typically 10 to 20 minutes and after 40 minutes the animal is indistinguishable from normal. This allows two successive doses of catechol to be measured with the drug to be tested for convulsant or anticonvulsant activity being administered in between. This model offers two main advantages over the traditional methods for detection of convulsant/anticonvulsant activity. Firstly since the entire procedure is carried out on surgically anaesthetized animals it is more humane than alternative model systems which use conscious animals. In addition the numbers of animals required is minimised by each animal acting as its own, no drug, control. This model system has, to date, been used for the detection of anticonvulsant activity by measuring the effect of anticonvulsants on the catechol-induced activity.[129] The anticonvulsants diazepam, sodium valproate and phenobarbitone significantly decreased the intensity of catechol-induced activity.



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4.1.2 Objectives

It was proposed to develop the anaesthetized mouse model as a means of detecting convulsant activity by assessing the ability of subconvulsant doses of known convulsants to potentiate catechol-induced convulsions. The model system was then to be used to assess the CNS effects of oxamniquine and its analogues by

measuring their effect on the catechol-induced convulsions in the anaesthetized mouse.

4.2 MATERIALS AND METHODS

4.2.1 Materials

(a) Animals

Male mice of the strain MF1, in the weight range 20 to 40g were used in the study.

(b) Drugs

Catechol, urethane, picrotoxin and strychnine hydrochloride were purchased from Sigma Chemical Co. Stock solutions of urethane 0.2 g/ml and strychnine 0.05 mg/ml were prepared in 0.9 %w/v saline and refrigerated. Picrotoxin was dissolved in normal saline containing a few drops of dilute hydrochloric acid to give a solution of concentration 0.25 mg/ml and refrigerated. Catechol solutions, 15 mg/ml in 0.9% w/v saline, were freshly prepared on each day and were protected from light as the solutions were noted to darken on exposure to light.

Oxamniquine, UK 4271 Lot 603/OX/701, was a generous gift from Pfizer Central Research, Sandwich, Kent, England.

Stock solutions of oxamniquine 15 mg/ml in citrate-phosphate buffer pH 5.0[130] were prepared and used in the tests requiring intravenous or intraperitoneal injection of the drug.

A 2.37 mg/ml dispersion of oxamniquine in 1 % Tween 80 was used in the experiments requiring oral administration of the drug. Since oxamniquine is available for therapeutic use only as an oral preparation, it was considered important to examine the effects of orally administered oxamniquine on catechol-induced convulsions to evaluate the central effects of the drug by this route.

The intravenous route of drug administration by-passes the membrane barriers which limit drug absorption after either oral, intramuscular, subcutaneous or intraperitoneal administration and delivers the drug directly into the systemic circulation. Bioavailability at the blood brain barrier is therefore not limited by absorption. Oxamniquine has been shown to attain significant levels in the brain after intravenous administration to female rats.[19] It was therefore considered important to examine the effects of intravenously administered oxamniquine on

catechol-induced convulsions in order to establish whether central stimulatory effects could be demonstrated.

The intraperitoneal route is the most commonly used route of drug administration in animal pharmacology. This route was chosen after it become apparent that the intravenous injection of a 60 mg/kg dose of oxamniquine was lethal.

6-Carboxy-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, the 6 carboxylic acid derivative (**9**) of oxamniquine and 6-methyl hydroxymethyl-2-N-isopropylaminomethyl-7-nitro-1,2,3,4-tetrahydroquinoline, the secondary alcohol derivative (**29**) were synthesized as described in Section 3.8 and 3.12 respectively. Stock solutions of the acid (**9**), as its hydrochloride salt, and the alcohol (**29**) were prepared in citrate-phosphate buffer pH 5.0 at concentrations of 7.5 mg/ml and 15 mg/ml respectively and used in the animal tests.

4.2.2 Methods

Spontaneous catechol-induced convulsions, occurring in the absence of intentional stimuli, were assessed by measuring total body activity of anaesthetized mice. Male mice were terminally anaesthetized with urethane (2 g/kg intraperitoneally) such that the righting reflex, corneal reflex and reflex retraction of hind-limb in response to a strong pinch on the toe pad were abolished. In certain mice, however, higher urethane doses, up to a maximum of 2.7 mg/kg, was required to achieve this degree of anaesthesia. Urethane was chosen as the anaesthetic because of its long-lasting stable depth of anaesthesia.

Total body activity was measured by placing the animals on their side in a plastic petri dish, suspended by cotton from a strain gauge attached via a transducer to a Washington recorder which recorded individual body twitches and jerks. The reaction apparatus, similar to one proposed by Angel[131] for the measurement of locomotor activity in anaesthetized animals is shown in Figures 4.1a and 4.1b. The animal's body temperature was maintained at approximately 37°C by suspending the dish over a water bath heated to 40°C.

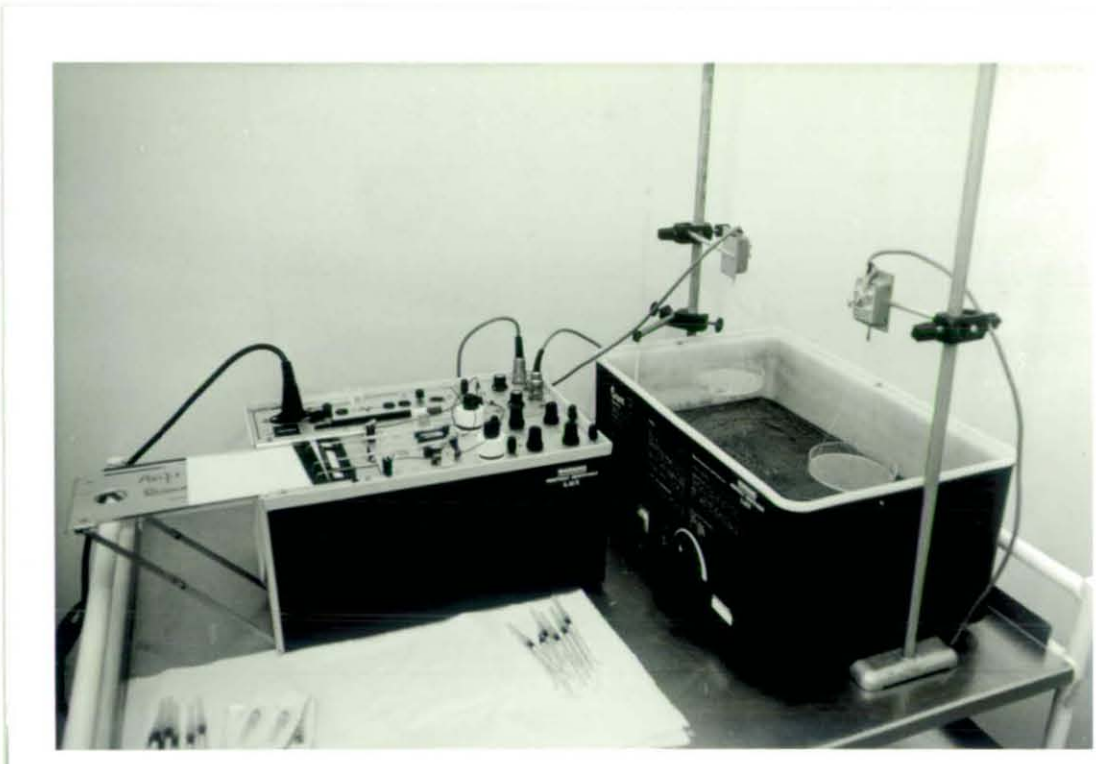


Figure 4.1a Apparatus used to measure total body activity.
A plastic petri dish was suspended by cotton from a strain guage which was attached via a transducer to aWashington recorder.

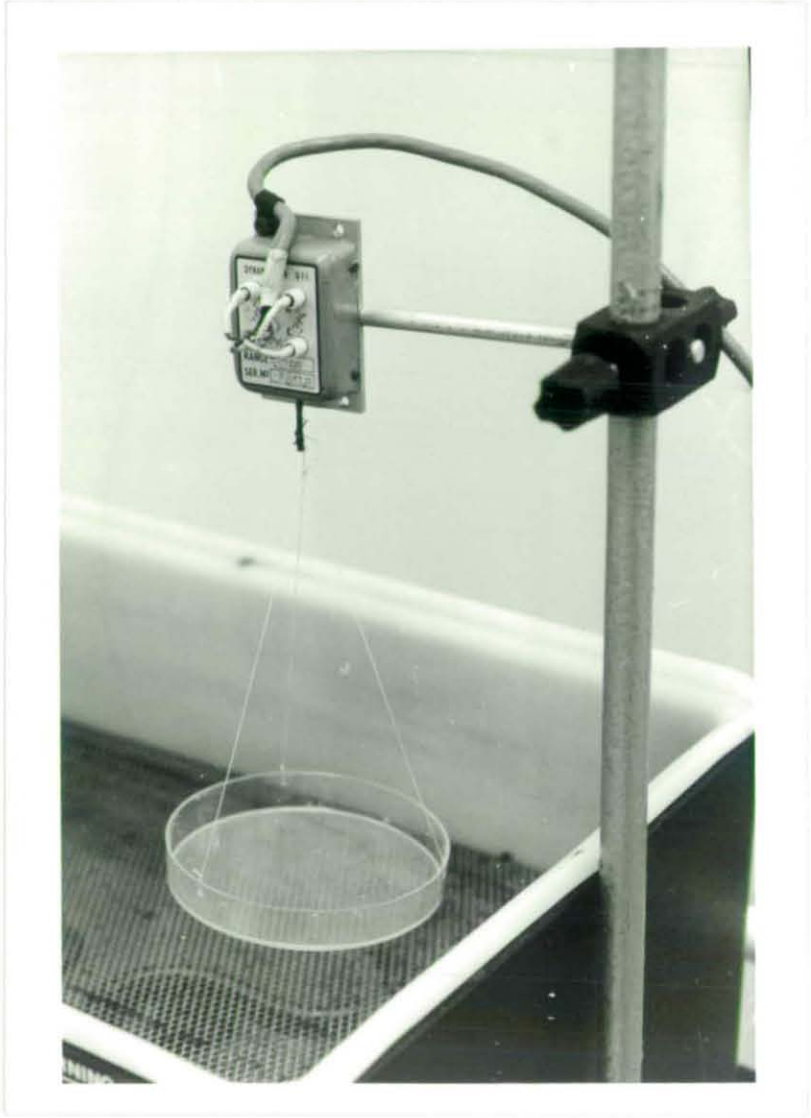


Figure 4.1b Apparatus used to measure total body activity.
The plastic container was suspended by cotton from a strain gauge attached to rod which was held firmly in a clamp.

The response of individual mice to an 80 mg/kg dose of catechol has previously been shown to be variable both in intensity and in duration.[128] This made it impossible to study the effects of drugs on catechol simply by comparing responses of control with those of drug treated mice. The same animal was therefore used as its own no drug control. The activity induced by catechol in the absence of the drug under test was recorded as the control response. Catechol-induced activity in the presence of the drug was then recorded in the same animal to assess the effect of the drug.

A typical experimental protocol (Fig. 4.2) involved recording basal activity for a 10 minute control period followed by injection of catechol and recording of total body activity for the following 20 minutes (C1). The second catechol dose was administered 90 minutes after the first and the activity induced recorded for a further 20 minutes (C2). With the exception of oxamniquine (30 mg/kg) which was administered intravenously, all the test drugs were administered intraperitoneally 90 minutes after the second dose of catechol. This was followed after a suitable time interval, either 20, 30 minutes or 60 minutes depending on the drug, by a third dose of catechol and the activity induced by this dose of catechol recorded over a 20 minute period (C3). The animal was however, observed over the entire 90 minute period after administration of the final catechol dose. Any activity induced by the test drug prior to administration of catechol was also recorded. This was taken to represent the inherent activity of the test drug on the anaesthetized mouse.

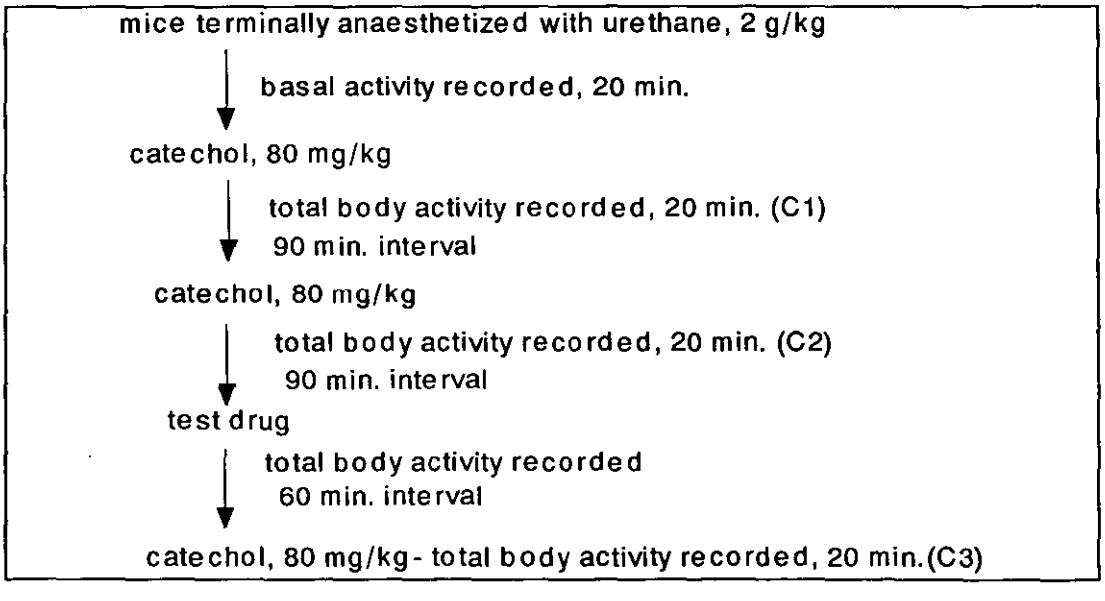


Fig. 4.2 Typical experimental protocol

Care was taken to maintain silence during the course experiments to avoid precipitation of myoclonic jerks by auditory stimulation.

The total body activity was recorded in arbitrary units by counting the individual jerks, recorded as spikes on the recorder tracing, over a 3 minute interval, during the time of maximum catechol-induced activity, typically 2 to 5 minutes after the dose. The percentage change in catechol-induced activity produced by the test drug was then calculated as follows:-

$$(C3 - C2)/C2\%.$$

This was taken as an index of the effect of the test drug on catechol-induced activity. Activity could not be recorded in absolute units because of the great individual variation in response to the same dose of catechol even within the same animal.

It was expected that potentiation of catechol-induced activity in the presence of the test drug would be represented by an increase in both the frequency as well as the duration of the convulsive episode. Conversely a decrease in the intensity, frequency and duration of catechol-induced activity would represent inhibition of catechol-induced activity. Qualitatively, the effect of the test drug on catechol-induced activity was expected to be indicated by the change in the amplitude of the twitches recorded. Drugs potentiating catechol activity would increased the amplitude. The converse would be true for those drugs which inhibit catechol activity.

A no drug, vehicle control was conducted for each drug to determine the effect of the vehicle on catechol-induced activity. In this set of experiments the test drug was replaced with a volume of the vehicle and the same experimental protocol was followed, the vehicle being administered between two consecutive doses of catechol.

The null hypothesis proposed for the study was that there is no difference between the response of the anaesthetized mouse to a convulsant dose of catechol in the presence of the test drug and in the presence of the vehicle in which the drug is dissolved, that is there is no difference in the response of the two populations to the same dose of catechol. Therefore the test drug has no effect on catechol-induced activity. The results were tested for statistical significance of the difference between the drug-treated and vehicle control groups by the Mann-Whitney U test,[132] a non-parametric test. A probability level of below 5% for the null hypothesis was considered statistically significant that is where the estimated

U value was smaller than the tabulated critical $U_{0.05}$ value for a two-tail test.[132,133] In such cases the test drug was considered to influence catechol-induced activity.

The mean percentage increase in catechol-induced activity and the standard error of the mean (SEM) were also calculated for both the drug-treated and control groups. However, it should be noted that this assumes a normal distribution of the population and this may not be the case in this particular study.

Only data from those animals surviving the effects of both the test drug and the subsequent catechol-induced convulsive activity was used in assessing whether the test drug had a significant effect on catechol-induced convulsions.

4.3 RESULTS

The results obtained indicated that an 80 mg/kg dose of catechol administered intraperitoneally produced activity in all animals and was sublethal. The convulsive episode, characterized by increased respiratory and heart rates, limb and body twitches and jerks and spontaneous convulsions, was induced within 2 minutes and lasted for 10 to 20 minutes. Maximum activity was attained between 2 and 6 minutes after administration of the dose. Several deaths were recorded in the course of the study and these will be discussed where relevant.

A typical tracing of the catechol-induced convulsive activity, as recorded by the Washington recorder is presented in Fig. 4.3.

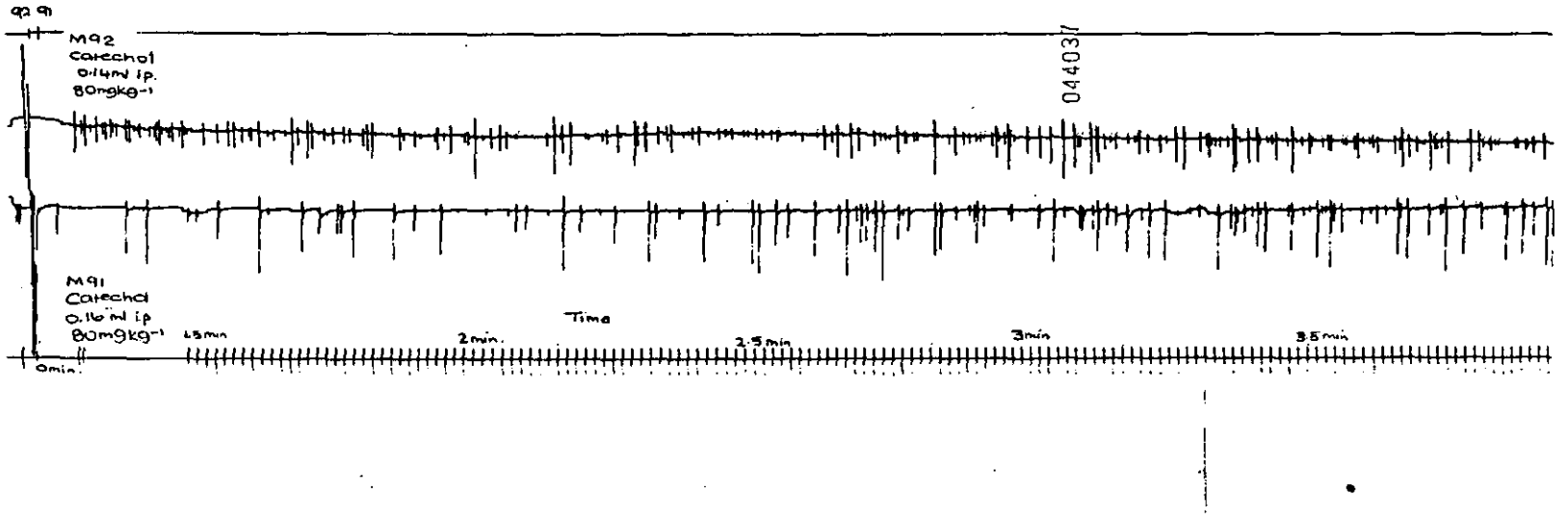


Figure 4.3 A typical tracing of catechol-induced activity
Catechol dose 80mg/kg i.p.
Attenuation: 0.1

The results obtained, represented as the percent change in catechol-induced activity, are presented in Tables 4.3 to 4.14.

4.3.1 The effect of strychnine on catechol-induced activity

The results of the effects of a subconvulsive dose of strychnine, 0.15 mg/kg, on the intensity and duration of catechol-induced activity are presented in Tables 4.3 and 4.4 respectively.

Table 4.3 The effect of strychnine, 0.15 mg/kg, on the intensity of catechol-induced convulsions

Strychnine, 0.15 mg/kg, ip			0.9% Normal Saline, 0.4 ml ip		
	% change	Rank		% change	Rank
M62	35	7	M68	25	4.5
M63	67	10	M69	25	4.5
M64	31	6	M70	-20	1
M65	45	8.5	M71	3	2
M66	116	12	M73	73	11
M67	239	13		Mean 21.2	
M68	17	3		SEM 34.4	
M69	45	8.5		n = 5	
	Mean 74.4	U' = 8			
	SEM 25.8	U =32			
	n = 8	$U_{0.05} = 6$			
		$U' > U_{0.05}$			

Table 4.4 The effect of strychnine, 0.15 mg/kg, on the duration of catechol-induced activity.

Strychnine, 0.15 mg/kg ip			0.9% Normal saline, 0.4 ml ip		
	% change	6		% change	Rank
M62	30	11	M68	33	7.5
M63	45	9.5	M69	0	3
M64	44	7.5	M70	15	4
M65	33	13	M71	25	5
M66	51	1	M73	48	12
M67	-8	9.5		mean 30.3	
M68	44	2		SEM 6.2	
M69	-4	U' = 16.5		n = 5	
	mean 29.4	U = 23.5			
	SEM 8.1	$U_{0.05} = 6$			
	n = 8	$U' > U_{0.05}$			

As can be seen from the tables the increase in the intensity and duration of catechol-induced activity observed when catechol was administered 30 minutes after the strychnine dose was not statistically significant, relative to the effects of normal saline, at the 95 % confidence limits. In both the test and the control experiments the catechol dose, 80 mg/kg, was administered 30 minutes after the test drug and saline control in both the test and control experiments respectively.

A study undertaken to determine the maximum subconvulsive dose of strychnine in the anaesthetized mouse, showed that a 1.5 mg/kg intraperitoneal dose was subconvulsive and produced non-lethal potentiation of catechol-induced activity. This dose produced no convulsions in the anaesthetized non-catechol treated animal. Although a 2.0 mg/kg dose of strychnine was found to be subconvulsant, this higher dose produced lethal potentiation of catechol-induced convulsions when administered intraperitoneally 30 minutes before the catechol dose.

The results of the effects of 1.5 mg/kg strychnine on the intensity of catechol-induced activity in the anaesthetized mouse are presented in Table 4.5.

Table 4.5 The effect of strychnine, 1.5 mg/kg, on the intensity of catechol-induced activity.

Strychnine 1.5 mg/kg i.p.			Normal saline, 0.4 ml i.p.		
	% change	Rank		% change	Rank
M102	234	8	M68	25	6
M103	625	12	M69	25	5
M104	643	13	M70	-20	2
M105	516	11	M71	3	4
M106	478	10	M73	73	7
M107	380	9	M106	-1	3
	mean 479	U = 0	M107	-100	1
	SEM 57	U' = 28		mean 0.714	
	n = 6	$U_{0.05} = 6$		SEM 0.27	
		$U < U_{0.05}$		n = 7	

Strychnine at a dose level of 1.5 mg/kg, administered intraperitoneally 30 minutes before a 30 mg/kg intraperitoneal dose of catechol, was found to potentiate the intensity of catechol-induced activity. This potentiation was significant at the 95 % confidence limits.

4.3.2 The effect of picrotoxin on catechol-induced activity

Preliminary investigations indicated that an intraperitoneal dose of picrotoxin, 2 mg/kg, administered 30 minutes before an 80 mg/kg dose of catechol was subconvulsive and potentiated, non-lethally, the intensity and duration of catechol-induced activity in the anaesthetized mouse. However, the results obtained were only qualitative and were not controlled for the effect of the vehicle.

4.3.3 The effect of oxamniquine on catechol-induced convulsions

(a) Effects of orally administered oxamniquine

Oral administration of the maximum total human dose of oxamniquine, 60 mg/kg, as a dispersion in 1 % Tween 80, was associated with the immediate induction of a hyperactive episode. This episode was characterized by increased breathing rate, increased heart rate, sporadic muscle jerks and some tremors typically lasting up to 8 minutes after drug administration after which normal basal activity was re-established and maintained until injection of the catechol dose. An increase in the intensity of catechol-induced activity was observed when the catechol dose was

injected 20 minutes after oxamniquine. However, when a 1 ml oral dose of 1 % Tween 80 was administered 20 minutes before the catechol dose a similar hyperactive episode was induced and subsequent injection of catechol was associated with an increase in the intensity of the resulting convulsive activity. Statistical evaluation of these results showed that there was no difference between the effects of oxamniquine and Tween 80 on catechol activity. The results are presented in Table 4.6.

Table 4.6 The effect of oral oxamniquine, 60 mg/kg, on the intensity of catechol-induced activity

Oxamniquine, 60 mg/kg, p.o.			1 % Tween 80, 1 ml, p.o.		
	% change	Rank		% change	Rank
M22	58	6	M32	638	10
M24	-100	1.5	M33	-100	1.5
M26	146	9	M34	16.4	4
M27	-67	3		mean 185	U = 11.5
M29	152	8		SEM 229.1	U' = 9.5
M30	35	5		n = 3	$U_{0.05} = 1$
M31	95	7			$U' > U_{0.05}$
	mean 46				
	SEM 37.2				

(b) The effects of intravenously administered oxamniquine.

The intravenous administration of a 60 mg/kg dose of oxamniquine dissolved in citrate-phosphate buffer pH 5.0, 15 mg/ml, resulted in death of the animals soon after drug injection. A 30 mg/kg dose of oxamniquine injected intravenously 20 minutes before an 80 mg/kg catechol dose increased the intensity, frequency and duration of catechol-induced activity. However, the intravenous administration of an equivalent volume of the drug vehicle to control animals, 20 minutes before the catechol dose induced similar effects. Statistical evaluation of the results showed that there was no significant difference between the effects of a 30 mg/kg dose of oxamniquine and those of an equivalent volume of the buffer on catechol-induced activity.

The results of the effect of oxamniquine on the intensity and the duration of catechol-induced activity are presented in Tables 4.7 and 4.8 respectively.

Table 4.7 The effect of oxamniquine, 30 mg/kg i.v., on the intensity of catechol-induced activity.

Oxamniquine, 30 mg/kg i.v.			Citrate-phosphate buffer pH5.0, i.v.		
	% change	Rank		% change	Rank
M37	**55**	**12.5**	**M37**	**55**	**12.5**
M40	**194**	**16**	M39	93	15
M42	**1**	**4.5**	M41	70	14
M44	33	9	M43	3	6
M45	19	8	M45	1	4.5
M46	16	7	M47	43	10.5
M47	43	10.5	M49	-66	2
M48	-36	3		mean 29.4	
M50	-78	1		SEM 20.2	
	mean 27.7	U' = 37		n = 7	
	SEM 24.9	U = 26			
	n = 9	$U_{0.05}$ = 12			
		U>U0.05			

Table 4.8 The effect of oxamniquine, 30 mg/kg i.v., on the duration of catechol-induced activity

Oxamniquine, 30 mg/kg i.v.			Citrate-phosphate Buffer pH 5.0, i.v.		
	% change	Rank		% change	Rank
M37	106	13.5	M37	106	13.5
M40	9	5	M39	400	16
M42	19	8	M41	135	15
M43	-3	2	M43	45	9.5
M44	-100	1	M45	45	9.5
M45	50	11	M47	0	3.5
M46	11	6	M49	0	3.5
M48	100	12		mean 104.4	
M50	14	7		SEM 52.8	
	mean 22.9	U'= 42.5		n = 7	
	SEM 20.3	U = 20.5			
	n = 9	U0.05 = 12			
		U>U0.05			

The 30 mg/kg intravenous dose of oxamniquine did not appear to have any inherent activity on the anaesthetized mouse. With exception of three cases, M37,

M40 and M42, this dose of oxamniquine did not induce any hyperactivity or other notable change in the behaviour of the mouse in the absence of catechol. In these three cases the administration of oxamniquine induced sporadic jerks of the limbs. This effect was of short duration, lasting up to 5 minutes after the injection, after which normal basal activity was re-established and maintained until intraperitoneal injection of the catechol dose. It may be important to note that the same degree of change in catechol-induced activity (55%) was observed in M37 when catechol was administered 20 minutes after either the oxamniquine or the buffer dose.

(c) The effects of intraperitoneally administered oxamniquine

In a preliminary study oxamniquine, 100 mg/kg, administered intraperitoneally 60 minutes before an intraperitoneal dose of catechol decreased the intensity of catechol-induced activity and the animals appeared to be very deeply anaesthetized. This dose of oxamniquine did not illicit any changes in the behaviour of the anaesthetized mouse in the absence of catechol. The basal rate was maintained until the catechol dose was administered.

The use of a higher intraperitoneal dose of oxamniquine, 200 mg/kg, was associated with further depression of catechol-induced activity. In 5 of the 7 cases recorded (71 %) the catechol-induced convulsions were completely abolished. Intraperitoneal injection of a 0.4 ml dose of the vehicle, citrate phosphate buffer pH 5.0, 60 minutes before the catechol dose potentiated catechol-induced activity. Statistical evaluation of the results showed that there was a significant difference between the effects of a 200 mg/kg intraperitoneal dose of oxamniquine and the effects of the citrate-phosphate buffer on catechol-induced activity.

The results of the effect of oxamniquine, 200 mg/kg, on the intensity and the duration of catechol-induced activity are presented in Tables 4.9 and 4.10 respectively.

Table 4.9 The effect of oxamniquine, 200 mg/kg i.p., on the intensity of catechol-induced activity

Oxamniquine 200 mg/kg i.p.			Citrate-phosphate buffer pH5.0, 0.4 ml i.p.		
	%change	Rank		% change	Rank
M56	-100	3	M94	-26	9
M57	-100	3	M95	44	12
M58	-85	6	M100	12	11
M59	-100	3	M101	0	10
M60	-100	3	M102	-47	8
M61	-56	7	M103	88	13
M87	-100	3	M104	234	15
	mean -91.6	U = 56	M105	141	14
	SEM 6.29	U' = 0		mean 56	
	n = 7	$U_{0.05} = 10$		SEM 33.4	
		$U' < U_{0.05}$		n = 8	

Table 4.10 The effect of oxamniquine, 200 mg/kg i.p. on the duration of catechol-induced activity.

Oxamniquine, 200 mg/kg i.p.			Citrate-phosphate buffer pH 5.0, 0.4 ml i.p.		
	% change	Rank		% change	Rank
M56	-100	3	M94	4.5	8
M57	-100	3	M95	-20.4	6
M58	17	10	M100	47.4	13
M59	-100	3	M101	73.9	15
M60	-100	3	M102	-5	7
M61	37	12	M103	29	11
M87	-100	3	M104	14.7	9
	mean -63.7	U = 37	M105	65.7	14
	SEM 23.5	U' = 9		mean 26.2	
	n = 7	$U_{0.05} = 10$		SEM 11.9	
		$U' < U_{0.05}$		n = 8	

The administration of oxamniquine, 200 mg/kg intraperitoneally, was associated with the induction of hyperactivity characterized by increased breathing and heart rates, strong neck contractions and extension of the body beginning within 60

seconds of the dose and lasting for about 4 minutes. Normal basal activity was then re-established and maintained until the next catechol dose. Two deaths were recorded within 60 minutes of the oxamniquine dose. In these two cases the initial hyperactivity induced by oxamniquine subsided to basal activity but later progressed into a hypoactive state resulting in death associated with respiratory difficulties.

4.3.4 The effect of the acid metabolite (9) on catechol-induced activity

The effect of a 140 mg/kg dose of the acid (**9**) equivalent to 70% of the oxamniquine dose shown to decrease catechol-induced activity was investigated.

The 140 mg/kg dose of the acid (**9**), injected intraperitoneally 60 minutes before the catechol dose, was found to increase the intensity of the catechol-induced activity. However, this increase in catechol-induced activity was not statistically significant when compared to the effect of the drug vehicle on catechol-induced activity. The drug vehicle, citrate-phosphate buffer pH5.0, (0.4 ml) was injected intraperitoneally 60 minutes before catechol. The results of the effect of this acid (**9**) on the intensity and duration of catechol-induced activity in the anaesthetized mouse are presented in Tables 4.11 and 4.12 respectively.

Table 4.11 The effect of the acid (**9**), 140 mg/kg i.p., on the intensity of catechol-induced activity

Acid (**9**), 140 mg/kg i.p.			Citrate-phosphate buffer pH5.0, 0.4 ml i.p.		
	% change	Rank		% change	Rank
M80	283	14	M94	-26	2
M81	27	5	M95	44	8
M82	93	10	M100	12	4
M83	33	6	M101	0	3
M84	**622**	**15**	M102	-47	1
M86	216	12	M103	88	9
M89	**1003**	**16**	M104	234	13
M91	41	7	M105	141	11
	mean 289.8	U = 15		mean 55.8	
	SEM 123.9	U' = 49		SEM 33.4	
	n = 8	$U_{0.05}$ = 13		n = 8	
		U > $U_{0.05}$			

Table 4.12 The effect of the acid (**9**), 140 mg/kg i.p., on the duration of catechol-induced activity

	Acid (**9**) 140 mg/kg i.p.			Citrate- phosphate buffer pH5.0 , 0.4 ml i.p.	
	% change	Rank		% change	Rank
M80	100	16	M94	4.5	4
M81	27	8	M95	-20.4	1
M82	50	11	M100	47.4	10
M83	16	7	M101	73.9	14
M84	61	12	M102	-5	3
M86	-12	2	M103	29	9
M89	93	15	M104	14.7	6
M91	6	5	M105	65.7	13
	mean 42.6	U = 24		mean 26.2	
	SEM 14.3	U' = 40		SEM 11.9	
	n = 8	U 0.05 = 13		n = 8	
		$U > U_{0.05}$			

Attention is drawn to the big increase in the intensity of catechol-induced activity observed after administration of the acid (**9**) to M84 and M89. In these two cases intraperitoneal administration of the acid (**9**) resulted in an instantaneous convulsive episode. The convulsive episode was characterized by strong rhythmic contractions of the body with neck and head jerks and full extension of the body and urination. The episode was of rapid onset, occurring within 30 seconds of injection of the acid, and lasted for about five minutes after which time normal basal activity was re-established and maintained until administration of the next catechol dose. A similar rapid onset convulsive episode was observed in several of the animals treated with an intraperitoneal dose of oxamniquine, 200 mg/kg. With the exception of M84 and M89, the acid (**9**) did not elicit any change in the behaviour of the animal in the absence of catechol.

The production of a yellow coloured urine by five of the animals within one hour of administration of the acid (**9**) was also noted.

Attempts to use higher doses of the acid (**9**), 240 mg/kg intraperitoneally, in order to evaluate the CNS activity of this compound were unsuccessful and resulted in death of the animals on administration of the catechol dose, 80 mg/kg, 60 minutes after the acid (**9**). Death was associated with respiratory difficulties.

4.3.4 The effect of the secondary alcohol (29) on catechol-induced activity

The effect of a 200 mg/kg dose of the alcohol (**29**) equivalent to the oxamniquine dose shown to decrease catechol-induced activity was investigated.

The intraperitoneal administration of a 200 mg/kg dose of the secondary alcohol (**29**), 60 minutes before the catechol dose, decreased the intensity and duration of catechol-induced activity. The alcohol (**29**) inhibited catechol-induced activity in the mouse. This inhibition was significant at the 95 % confidence limits when compared with the effect of a 0.4 ml dose of the buffer on catechol activity.

The results of the effect of the alcohol (**29**) on the intensity and duration of catechol-induced convulsions are presented in Tables 4.13 and 4.14 respectively.

Table 4.13 The effect of the alcohol (**29**), 200 mg/kg i.p., on the intensity of catechol-induced activity

Secondary alcohol (**29**), 200 mg/kg i.p			Citrate-phosphate buffer pH5.0, 0.4 ml i.p.		
	% change	Rank		% change	Rank
M74	-100	2.5	M94	-26	6
M75	-100	2.5	M95	44	10
M76	-100	2.5	M100	12	8
M79	**38**	**9**	M101	0	7
M92	-100	2.5	M102	-47	5
	mean -78	U = 36	M103	88	11
	SEM 27.6	U' = 4	M104	234	13
	n = 5	$U_{0.05} = 6$	M105	142	12
		$U' < U_{0.05}$		mean 56	
				SEM 33.4	
				n = 8	

Table 4.14 the effect of the alcohol (**29**), 200 mg/kg i.p., on the duration of catechol-induced activity

Secondary alcohol (**29**), 200 mg/kg i.p			Citrate-phosphate buffer pH 5.0, 0.4 ml i.p.		
	% change	Rank		% change	Rank
M74	-100	2.5	M94	4.5	7
M75	-100	2.5	M95	-20.4	5
M76	-100	2.5	M100	47.4	10
M79	94	13	M101	73.9	12
M92	-100	2.5	M102	-5	6
	mean -61.2	U = 32	M103	29	9
	SEM 38.8	U' = 8	M104	14.7	8
	n = 5	$U_{0.05} = 6$	M105	65.7	11
		$U' > U_{0.05}$		mean 26.2	
				SEM 12	
				n = 8	

The alcohol (**29**) did not display any inherent activity and did not elicit any activity in the absence of catechol. The animals appeared to be very deeply anaesthetized after injection of the alcohol and the basal rate was maintained in all cases. In all but one case, M79, the alcohol (**29**) completely abolished catechol-induced activity and injection of the catechol dose did not induce a convulsive state. Two deaths occurring within 30 minutes of the administration of the alcohol (**29**) were recorded, these are not included in the results presented. Other general effects, including urination, were not observed in the animals after dosing with the alcohol (**29**) even in the presence of catechol.

A summary of the results obtained in the study is presented in Table 4.15.

Table 4.15 Summary of the effect of the test drugs on the intensity of catechol-induced activity in the anaesthetized mouse.

Drug	Dose	Effect on catechol-induced activity (relative to vehicle control)	Number
Strychnine	0.15 mg/kg, i.p.	not significant	8
Strychnine	1.5 mg/kg, i.p.	increase	6
Oxamniquine	60 mg/kg, o.p.	not significant	7
Oxamniquine	30 mg/kg, i.v.	not significant	9
Oxamniquine	200 mg/kg, i.p.	decrease	7
Acid (**9**)	140 mg/kg, i.p.	not significant	8
Alcohol (**29**)	200 mg/kg, i.p.	decrease	5

4.4 DISCUSSION

The convulsant screen

In agreement with the results obtained by Angel[128] the 80 mg/kg dose of catechol produced activity in all animals and was sublethal. However, although Angel reported that the effects of catechol were completely eliminated within 40 minutes of the dose, we noted residual effects of catechol, manifested as hyperactivity on the administration of subsequent doses of catechol/test drugs. The dose interval between consecutive catechol doses was therefore increased to 90 minutes to ensure complete elimination of previous dose of catechol before further doses were administered.

The convulsions induced by catechol in the anaesthetized mouse resemble human epilepsy because they can be antagonized by drugs used in the treatment of human epilepsy. The model has previously been used to detect anticonvulsant activity.[129] Diazepam, phenobarbitone and sodium valproate significantly decreased the intensity of catechol-induced activity. The model is therefore a potential *in vivo* model of epilepsy for the screening of antiepileptic drugs. In the present study it was demonstrated that in addition to detecting anticonvulsant activity, the model is able to detect convulsant activity. Catechol-induced activity was enhanced in the presence of the convulsant agent strychnine. A 1.5 mg/kg dose of strychnine, administered intraperitoneally to the anaesthetized mouse was shown to potentiate the convulsant activity of an 80 mg/kg dose of catechol administered 30 minutes later. This dose of strychnine was subconvulsant and did

not induce convulsions in the anaesthetized non-catechol treated mouse. Potentiation of catechol-induced convulsant activity by a subconvulsant dose of strychnine confirmed that the model is able to detect convulsant activity. Therefore in addition to screening for anticonvulsant activity the model may also be used for the screening of drugs known to induce convulsions as a side effect or for the screening of new compounds for possible convulsant activity.

Further work using other standard convulsant agents needs to be done to confirm that the model is capable of detecting convulsant activity irrespective of its aetiology. The effects of standard convulsants including pentylenetetrazole and picrotoxin need to be evaluated. Pentylenetetrazole is the most commonly used systemic chemical convulsant for the induction of epileptiform seizures in experimental models of epilepsy. It would be useful to compare the effects of strychnine and pentylenetetrazole on catechol-induced activity as the convulsions induced by strychnine are not epileptiform but consist of tonic extensor jerks which may be triggered by any sensory stimulus.[124]

The use of the above model to evaluate the CNS effects of oxamniquine was justified by similarities between oxamniquine-induced seizure activity and human epilepsy.[39,40,42] which are as follows:

(a) Oxamniquine-induced seizure activity responds to the administration of intravenous diazepam and the use of anticonvulsants prior to the initiation of treatment with oxamniquine, especially in patients with a history of convulsive disorder, to prevent the occurrence of the convulsive episode has been advocated.[8,39,40]

(b) Oxamniquine-induced seizure activity manifests the two criteria essential for classification of a seizure as epileptic-like: (i) epileptic-like activity in the EEG and (ii) clinical seizure-like activity manifested by motor movements or behavioural changes.[125] In those patients suffering oxamniquine induced seizures, seizure activity was accompanied by EEG abnormalities characteristic of idiopathic epilepsy.[40]

The use of oxamniquine in patients with a history of epilepsy is contraindicated.[8]

Effects of oxamniquine on catechol-induced activity

It was initially proposed to evaluate the epileptogenic potential of oxamniquine using three dose levels: (i) 60 mg/kg, the maximum human dose (ii) 180 mg/kg, three times the maximum dose and (iii) 600 mg/kg, ten times the maximum dose. The use of doses far in excess of the therapeutic dose level was advocated since the study was intended to demonstrate a toxic manifestation of low incidence in

the population. A large dose was therefore required to increase the probability of demonstrating the side effect. It was found however, that the aqueous solubility of oxamniquine severely limited the maximum dose that could be administered. In the original toxicological studies of oxamniquine evaluated the effects of oral and intramuscularly administered suspensions of oxamniquine in experimental animals.[32,33] Convulsions were reported to occur in some rodents dosed with the drug however, details of the dose or dosage regimen used were not reported.

Oral administration of a 1 ml dose of oxamniquine, 60 mg/kg dispersion in 1% Tween 80, was associated with the induction of a hyperactive episode on drug administration and an increase in the intensity of catechol-induced activity. This effect was however, considered as a non specific false positive response since oral administration of a 1 ml dose of the vehicle, 1% Tween 80, was associated with the induction of a similar episode. There was no difference between the effects of the two treatments on catechol-induced activity at the 95 % confidence limits. It was thought that the disturbances associated with handling the animal during administration of the oral dose may be responsible for the hyperactive response observed. Although the intubation of animals to facilitate oral drug administration with minimal handling has been used in some animal experiments, this approach was not considered feasible in the present study. It is possible that the presence of the gastric tube may alter the behaviour of the animal. The screen was based on the detection of total body activity. It was therefore essential that external influences be kept to a minimum as they may cause a change in body activity which would be recorded as a drug-induced effect. Since oral administration of oxamniquine was associated with non specific effects and the drug is poorly and erratically absorbed by the intramuscular route,[32] the effects of intravenously and intraperitoneally administered oxamniquine on catechol-induced activity was investigated.

The evaluation of the effects of an intravenous dose of oxamniquine on catechol-induced activity has precedence in the work reported by Kokwaro and Taylor.[18] Although it was reported that significant levels of oxamniquine in the brain were attained within 1 hour of an intravenous dose of oxamniquine (15 mg/kg) to the rat, the CNS activity of oxamniquine in doses comparable to human therapeutic doses (15-60 mg/kg) could not be demonstrated in the anaesthetized mouse. The intravenous injection of oxamniquine, 30 mg/kg, had no significant effect on convulsions induced by catechol in the anaesthetized mouse. It was concluded that this dose of oxamniquine, 30 mg/kg, had no detectable central effect in this model. Attempts to investigate the effects of a higher oxamniquine dose were

unsuccessful. The intravenous administration of a 60 mg/kg dose of oxamniquine was fatal and the animals died soon after dosing. It is possible that the administration of the oxamniquine dose as a large bolus may have adverse effects on the cardiac system or that the drug may precipitate out of solution resulting in fatal embolism. The effect of higher doses of oxamniquine on catechol-induced activity was investigated using oxamniquine doses administered intraperitoneally.

Oxamniquine, in doses of 100 mg/kg and above, depressed catechol-induced activity when administered intraperitoneally 60 minutes before the catechol dose. A 200 mg/kg dose of oxamniquine depressed both the intensity and the duration of catechol-induced convulsions. This effect which was shown to be significant at the 95 % confidence levels. A CNS depressant activity was demonstrated with doses of oxamniquine higher than the therapeutic dose. This depressant activity was in contrast to the expected CNS excitatory activity. However, since catechol is known to produce its convulsant activity by a central mechanism,[128] the inhibition of this activity by oxamniquine demonstrated that the CNS depressant activity of oxamniquine is central in origin.

Although it was anticipated that the screen would demonstrate the epileptogenic activity of oxamniquine, the demonstration of a CNS depressant activity was not altogether surprising as drowsiness is the most commonly encountered side effect of oxamniquine in clinical use (50.6%).[41] This is in contrast to the convulsant effects of oxamniquine which are of low incidence (1.1%).[41]

It is possible that the CNS effects of oxamniquine resemble those of ethanol. Ethanol is known to cause either CNS depression or excitation depending on which CNS centres are inhibited first. Depression of the inhibitory centres gives rise to central excitation while depression of the excitatory centres results in central depression. A similar biphasic reponse is also observed with general anaesthetics which cause excitation at low doses and depression at higher doses. It is possible that a similar mechanism may be operational in the case of oxamniquine.

Although the 200 mg/kg dose of oxamniquine did not potentiate catechol-induced activity in the anaesthetized mouse, administration of the oxamniquine dose was associated with the instantaneous induction of a hyperactive episode characterized by increased breathing and heart rates as well as rhythmic contractions of the body and jerks. The episode was of rapid onset (within 60 seconds) and short duration (4 minutes) after which basal activity was re-established. The origin and

significance of this hyperactive episode was difficult to establish as the induction time was too short to have allowed systemic absorption and partitioning of the drug into the CNS.

It is important to note that the aim of this study was to establish whether the effects of oxamniquine on the CNS could be demonstrated in an *in vivo* model. Therefore it was imperative that adequate levels of the drug be attained in the systemic circulation so that equilibration at the blood brain barrier (BBB) would not be limited by lack of bioavailability the drug at this membrane. The intravenous route by-passes the barriers to absorption normally encountered after either oral, intramuscular, or intraperitoneal administration and delivers the drug directly into the systemic circulation so that maximum blood levels are attained. In the case of oxamniquine circumvention of the gut/blood barrier was particularly important as the drug is subject to presystemic metabolism by enzymes in the gut wall.[16] Individual variation in presystemic metabolism may account for the individual variation in serum concentration and time to peak observed in human pharmacokinetic studies.[17] Thus although it has been shown in the present study that intraperitoneal administration of a 200 mg/kg dose of oxamniquine had a CNS depressant effect, it is debatable whether the effects observed in this study are truly representative of the clinical situation since intraperitoneal administration by-passes the barrier to oral absorption as well as presystemic metabolism in the gut wall. The oxamniquine levels attained after intraperitoneal administration are therefore much higher than would otherwise be attained after oral administration.

Notwithstanding the foregoing, it is important that the ability of oxamniquine to induce CNS depression by a central mechanism has been demonstrated. Similarly the demonstration of the ability of oxamniquine to penetrate the BBB and attain brain levels higher than plasma levels within 1 hour,[18] intravenous drug administration to the rat is not representative of the clinical situation. In human pharmacokinetic studies, oxamniquine was shown to be extensively metabolized to acidic metabolites after oral administration. At the time of peak serum concentrations, 0.5 to 3 hours after dosage, only 10 % of the drug related material was comprised of the unchanged drug.[15] As oxamniquine is available for therapeutic use only in an oral dosage form it is suggested that in clinical use, oxamniquine can elicit central effects only if sufficiently high concentrations can be attained in the brain after oral administration.

Further studies are required to evaluate the absorption profile of oxamniquine in the mouse after intraperitoneal administration. The peak blood levels, the time to peak as well as the brain levels attained after intraperitoneal administration need to be determined so that the CNS effect may be correlated with drug levels in the brain. In the present study none of these parameters were known. The 60 minute dose interval between oxamniquine and catechol was based on the 60 minute interval within which maximum brain levels of oxamniquine were attained after intravenous injection to rats. It was estimated that the absorption rate after intraperitoneal administration in the mouse would be comparable to the absorption rate after intravenous dosage in the rat since the mouse has a larger absorption surface area relative to its size. The use of slow intravenous infusion to administer the oxamniquine dose as well as the effect of other doses of oxamniquine on catechol-induced activity needs to be evaluated. The plasma concentrations of oxamniquine attained in each case would have to be determined.

The effects of the acid (9) on catechol-induced activity

The acid (**9**) is the major metabolite of oxamniquine in mammals. Although the fact that the acid (**9**) is devoid of schistosomicidal activity is well documented, the biological activity and pharmacology of this compound in mammals does not appear to have been reported. It was the intention of this study to evaluate the central effects of the acid (**9**) to determine its contribution to the central effects of oxamniquine observed in clinical use. It is however, debatable whether significant levels of the acid (**9**) in the CNS may be attained after therapeutic administration of an oral dose of oxamniquine due to the hydrophilic nature of the acid. The relevance of any results obtained in the study to the clinical situation is therefore questionable. Notwithstanding this, it was important to ascertain the possible role of the acid (**9**) in the aetiology of the central effects of oxamniquine.

CNS activity attributable to the acid (**9**) was not, however, demonstrated in the study. There was no significant difference between the effect of an intraperitoneal dose of the acid (**9**) and the influence of the vehicle on catechol-induced activity in the anaesthetized mouse. Pharmacokinetic parameters such as absorption of the acid (**9**) into the systemic circulation, partitioning across the BBB and distribution in the CNS versus elimination of this water soluble compound may account for the failure to demonstrate any central activity. A hyperactive episode similar to that induced by oxamniquine was, however, illicited in two animals soon after administration of the acid (**9**). The induction of

this convulsive episode may be significant since elevated responses to catechol-induced activity was observed in these two animals.

The dose of the acid (**9**), 140mg/kg, 70 % of the oxamniquine dose shown to have a significant CNS depressant effect was chosen on the basis that 41 to 73% of the oxamniquine dose is excreted as the 6-carboxylic acid metabolite.[21] It was thought that the use of a metabolite dose that was 70 % of the oxamniquine dose would be an approximation of the metabolite concentrations attained after administration of oxamniquine. It is, however, questionable whether this was a justifiable assumption as the blood levels of the acid (**9**) after intraperitoneal administration of oxamniquine administration are not known. Attempts to demonstrate the CNS activity of the acid (**9**) using a higher dose, 280 mg/kg, were unsuccessful and resulted in death of the animals on administration of the catechol dose 60 minutes later.

The blood level profile of the acid (**9**) after intraperitoneal administration needs to be evaluated. This is important because it would give details of the blood levels attained as well as the time to peak and therefore the systemic bioavailability of the acid (**9**) after intraperitoneal administration. The time of peak blood levels is important so that the catechol dose can be administered to coincide with the time of peak levels of the acid in the systemic circulation. In addition the effects of an intravenous dose of the acid (**9**) on catechol-induced activity needs to be investigated. Intravenous administration of the acid may eliminate the complications presented by poor systemic bioavailability of the acid (**9**) after intraperitoneal administration.

In *in vivo* studies absorption, distribution, metabolism and excretion influence the efficacy of a given drug. The use of a local model *e.g.* local application of the acid (**9**) to isolated parts of the brain may be able to unequivocally establish whether the acid (**9**) does indeed have any central effects. Such a model would eliminate the complicating factors of absorption, distribution, metabolism and excretion. The demonstration of central effects of the acid (**9**) in such a model would, however, indicate the potential of the acid (**9**) to induce such central effects in man only if significant concentrations of the acid (**9**) could be attained in the CNS.

In general lipophilic drugs are better absorbed than non-lipophilic ones. The concept of penetration of biological membranes only by non-ionized species is, however, an oversimplification of a more complex phenomenon which may include transportation of an ion pair species in certain cases.[134,135] Ion pairs may

frequently complicate the relationship between lipophilicity and drug transport and the possibility that the acid (**9**) may penetrate the BBB if adequate blood levels could be attained should not be discounted. Furthermore, the acid (**9**) contains two basic NH groups in addition to the acid functionality. It is possible that the acid (**9**) may exist as a zwitterion at physiological pH. The predominant species of the acid (**9**) at physiological pH therefore needs to be determined. It would also be important to determine the distribution coefficient (Log D) for the acid (**9**) so that its biological distribution could be predicted. The use of distribution coefficients for the analysis of structure-activity relationships of ionizable compounds has been described.[135]

Effects of the secondary alcohol (29) on catechol-induced activity

The CNS depressant activity of the alcohol (**29**) was demonstrated in the study. Intraperitoneal administration of a 200 mg/kg dose of the alcohol (**29**) inhibited catechol-induced activity and in all but one case the activity was completely abolished. The animals appeared to be very deeply anaesthetized after intraperitoneal administration of the alcohol (**29**). It is possible that the alcohol (**29**) may have a very potent depressant effect on the animals and may potentiate the anaesthetic resulting in an even greater degree of anaesthesia.

The alcohol (**29**) is the one carbon homologue of oxamniquine and may be expected to be more lipophilic. The octanol-water Log P value for oxamniquine, 2.245,[19] is in the range of neutral compounds which readily penetrate the CNS. The alcohol (**29**) may have a Log P value in the same range and may therefore penetrate the CNS. The deeper degree of CNS depression obtained with the alcohol (**29**), relative to that induced by oxamniquine, may be an indication that higher levels of the alcohol (**29**) are attained in the CNS. It would be useful to determine the pKa's as well as the octanol-water partition coefficient for the alcohol (**29**) so that the hydrophobicity parameter, Log P, may be calculated and compared with the value for oxamniquine. This would enable the membrane penetration characteristics, biological distribution and bioavailability of the alcohol (**29**) to be predicted and would give some indication of the potential biological activity. Comparison of the Log P values of oxamniquine and the alcohol (**29**) would give some indication of the relative levels of each drug attained in the CNS.

The present study demonstrated that the alcohol (**29**) has CNS depressant activity similar to that observed with oxamniquine. However, it is not known whether it is the intact alcohol (**29**) or some metabolite which elicits this activity.

Further studies are required to evaluate the absorption profile of the alcohol (**29**) in the mouse after intraperitoneal administration. The peak blood levels, the time to peak as well as the brain levels attained after intraperitoneal administration need to be determined so that the CNS effects observed may be correlated with drug levels in the brain. The biological activity of the alcohol (**29**) also needs to be evaluated. It was anticipated that the potential biological activity of the alcohol (**29**) would be established, with reference to oxamniquine, in a study of the effects of the alcohol (**29**) on RNA biosynthesis *in vitro*.

Conclusion

The study demonstrated that convulsant activity can be evaluated by examining the effects of convulsant drugs on catechol-induced activity in the anaesthetized mouse. A subconvulsant dose of strychnine, 1.5 mg/kg administered intraperitoneally, potentiated the convulsive episode induced by catechol. This observation confirmed that the model is capable of detecting convulsant activity. This model has previously been used to evaluate anticonvulsant drugs. It is suggested that the model offers the following advantages over other models for the detection of convulsant/anticonvulsant activity:

(i) the model uses unconscious animals and is therefore more humane,
(ii) fewer numbers of animals are required because each animal acts as its own no drug control and
(iii) the model requires simple apparatus and gives rapid results.

The short comings of the model include:

(i) only acute studies are possible with this model, the model is not suitable for chronic studies which are required for the evaluation of anticonvulsant drugs due to the chronic nature of epilepsy which requires long term treatment,
(ii) the effects of the anaesthetic on the drug are not known and
(iii) the model is an *in vivo* model and is therefore complicated by the effects of absorption, distribution, metabolism and excretion.

The effects of oxamniquine (**1**), the 6-carboxylic acid derivative (**9**) and the secondary alcohol derivative (**29**) on the CNS were evaluated using the *in vivo* screen. The screen involved the evaluation of the effect of the drugs on catechol-induced convulsant activity in the anaesthetized mouse. The intraperitoneal administration of oxamniquine and the secondary alcohol (**29**), at a dose level of 200 mg/kg, were shown to significantly decrease both the intensity and duration of the convulsant activity induced by a dose of catechol administered 60 minutes after the test drug. The reduction in convulsant activity was significant at the 95%

confidence level and was taken to represent CNS depressant activity of central origin. The alcohol (**29**) was shown to be a more potent depressant than oxamniquine and completely inhibited catechol-induced convulsions in all but one of the animals used in the screen. The acid (**9**) was shown to be devoid of any central activity in the model at a dose level of 140 mg/kg. Intraperitoneal administration of a 140 mg/kg dose of the acid (**9**) had no effect on catechol-induced activity. This lack of central activity may, however, arise as a result of poor systemic absorption and bioavailability of this acid at the BBB due to its very hydrophilic nature. Rapid elimination of the acid (**9**) with minimal absorption may preclude the attainment of adequate blood levels.

As noted above, the use of an *in vivo* model is complicated by the effects of absorption, distribution, metabolism and excretion as well as the role secondary metabolites and problems associated with defining the drug's site of action when the drug is administered systemically. The use of focal microinjections of the drug may unequivocally establish whether oxamniquine and/or its analogues possess any epileptogenic activity.

A less lipophilic derivative of oxamniquine which is still able to be absorbed systemically and which also retains the schistosomicidal activity would offer the advantages of oxamniquine with decreased CNS effects.

CHAPTER FIVE

A PRELIMINARY EVALUATION OF THE EFFECTS OF OXAMNIQUINE AND ANALOGUES ON RIBONUCLEIC ACID BIOSYNTHESIS IN VITRO

5.1 INTRODUCTION

The aim of this work was to investigate the effects of oxamniquine and some of the novel analogues synthesized on *in vitro* ribonucleic acid (RNA) biosynthesis in order to establish the potential schistosomicidal activity of these analogues.

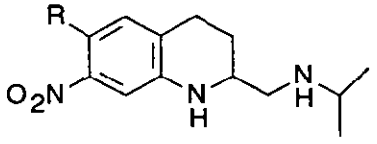
5.1.1 Background

Oxamniquine is a potent schistosomicide active principally against *S. mansoni.* As noted previously in Chapter One Section 1.3 the specific aims of the project were firstly, to synthesize structural variants of oxamniquine and secondly, to evaluate the effect of structural modification on the biological activity, in particular any central nervous system (CNS) activity.

The synthesis of the acid (**9**), the alcohol (**29**) and the diol (**31**), three of the compounds proposed as synthetic target compounds, is reported in Section 2.2.4., Section 2.2.6. and Section 2.2.7.(b) respectively.

The evaluation of the effects of the oxamniquine (**1**), the acid (**9**) and the alcohol (**29**) on the CNS is reported in Chapter Four. The CNS depressant effect of oxamniquine and the alcohol (**29**), administered intraperitoneally as a 200 mg/kg dose, was demonstrated in the anaesthetized mouse model.[128,129] CNS effects attributable to the acid (**9**) were not, however, demonstrated in this model.

It was therefore important to evaluate the potential schistosomicidal activity of the novel analogues of oxamniquine, the alcohol (**29**) and the diol (**31**), in order to establish whether structural modification of the oxamniquine molecule resulted in the formation of compounds with oxamniquine-like activity. The acid (**9**) is the major metabolite of oxamniquine in mammals and has been reported to be devoid of schistosomicidal activity.[22]



R	
CH_2OH	(**1**)
$CH(OH)CH_3$	(**29**)
$CH(OH)CH_2OH$	(**31**)

(a) Screening procedures for antischistosomal activity

Screening of compounds for antischistosomal activity involves the exposure of the parasite, the schistosome worm, to the drug compound either *in vitro* or *in vivo*. The effects of the compounds on the motility and/or survival of the worms after exposure to the drug are used as an indication of potential antischistosomal activity.

The classical procedures used for the screening of drug compounds for antischistosomal activity have been reviewed by Sturrock *et. al.*[136] Compounds synthesized as potential schistosomicides are subjected to *in vivo* screening initially in primary screens to evaluate their schistosomicidal activity and then in secondary screens to evaluate possible efficacy in man.

Generally rodents, mice or hamsters, habouring an experimental infection are used in the primary screen. Detection of therapeutic schistosomicidal activity may be indicated by (i) the elimination or reduction of faecal egg excretion and (ii) reduced mortality in treated, infected mice compared to untreated, infected controls. The demonstration of the reduction or elimination of the adult worm burden in drug treated, infected animals compared to untreated, infected controls is the most conclusive indication of potential schistosomicidal activity. Primary screens are also used to evaluate the activity of structural analogues of the active compound.

Secondary evaluation of promising compounds in a primate species is an essential step in the investigation since confirmation of schistosomicidal activity in this higher species would indicate possible efficacy in man. Secondary screens are also used to establish suitable doses for clinical trials and to detect harmful side effects. Many subhuman primates are susceptible to human schistosomiasis although the infection may not follow an identical course to that in humans. Baboons and monkeys are the main candidates used as subhuman primate models for drug trials. Efficacy of the drug is judged either by: (i) reduction in egg load, treatment is considered curative when the count falls to zero and remains so for several weeks, or (ii) adult worm recoveries and tissue egg counts at autopsy.

However, these screening methods are expensive and involve the use of special handling procedures and facilities to maintain viable *S. mansoni* cultures. A rapid and inexpensive screen was required for preliminary evaluation of the potential schistosomicidal activity of the oxamniquine analogues. It was therefore proposed

to use a screening method based on the mode of action of oxamniquine. Only those compounds showing promising activity in the preliminary screen would be submitted to specialized laboratories for screening against the schistosome worm.

(b) Mode of action of oxamniquine

As alluded to in Chapter One (Section 1.1.3e), the mode of action of oxamniquine is thought to be via alkylation of the deoxyribonucleic acid (DNA) of schistosomes sensitive to the drug. It has been proposed that oxamniquine is metabolically activated *in vivo* to a reactive ester which acts as an alkylating agent and binds covalently to schistosomal DNA.[48] A schematic representation of the mode of action is given in Scheme 1.1. page 14. It has been suggested that extensive alkylation of DNA may interfere with DNA replication and RNA biosynthesis resulting in inhibition of macromolecule biosynthesis culminating in worm death.[47]

Studies on the mode of action of oxamniquine have demonstrated the binding of triturated oxamniquine to sensitive schistosome DNA *in vitro*.[51] Studies have also shown that oxamniquine is able to inhibit the incorporation of triturated uridine into hycanthone/oxamniquine-sensitive *S. mansoni*.[49] A good correlation was obtained between schistosomicidal activity of the hycanthone/oxamniquine family and early irreversible inhibition of schistosome-uridine incorporation.[49] However, oxamniquine binding to DNA has only been demonstrated in the schistosome worm.[51] Previous studies have failed to demonstrate the binding of oxamniquine to calf thymus DNA using the flow dialysis method,[50] an *in vitro* laboratory model of DNA binding. This is in agreement with the proposal that bioactivation of oxamniquine is an essential prerequisite for its schistosomicidal activity. Therefore the use of reagents commonly used to estimate alkylating activity *e.g.* 4-(4-nitrobenzyl)pyridine[137] as a rapid qualitative screen was not possible.

Recent studies have shown that oxamniquine is able to irreversibly inhibit *in vitro* RNA biosynthesis in a mammalian system.[50] The potent schistosmicides: oxamniquine (**1**), praziquantel (**6**) and hycanthone (**3**) inhibited the incorporation of triturated-uridine into macromolecules of isolated hamster liver nuclei, a model of *in vitro* RNA biosynthesis. The irreversible inhibition of RNA biosynthesis by oxamniquine demonstrated in this model suggested that the mechanism of inhibition may be via covalent modification of some component of the RNA biosynthesis machinery.[50] This is in agreement with the formation of a drug-DNA adduct, on metabolic activation of oxamniquine, which has been proposed as a possible mechanism for the mode of action of oxamniquine.

5.1.2 Rationale

It was therefore proposed to evaluate the effects of oxamniquine and the analogues, the alcohol (**29**) and the diol (**31**), on *in vitro* RNA biosynthesis using isolated hamster liver nuclei. It was anticipated that a comparison of the effects of the oxamniquine analogues on RNA biosynthesis with that of oxamniquine would give some indication of the potential schistosomicidal activity of the analogues.

This model had the added advantage that the schistosomicidal potential of the analogues could be evaluated in an *in vitro* system without using the schistosome worm. However, demonstration of inhibition of RNA biosynthesis in this model would only be a preliminary indication of possible schistosomicidal activity which would have to be confirmed by evaluating the effects of the compounds on the schistosome worm.

It should be noted that the study was intended to be a screen for biological activity and not a mode of action study.

The effect of drugs on RNA biosynthesis *in vitro* may be evaluated by assessing the ability of the drug to interfere with the incorporation of radiolabelled uridine into macromolecules.[138] Functionally active isolated cell nuclei provide a system intermediate between intact cells and in vitro RNA synthesis from a chromatin template with isolated RNA polymerase.[139] After incubation of the nuclei with the drug in the presence of radiolabelled uridine, the macromolecule precursor, the macromolecules are isolated and liquid scintillation spectroscopy is performed. The degree of synthesis is inversely proportional to the amount of radioactivity associated with the isolated macromolecules.

Isolated nuclei provide an opportunity not only to study transcription of defined RNA species but also the intranuclear metabolism and eventually the release of the RNA.[139]

5.1.3 Objectives

The objectives of the study were firstly, to demonstrate the inhibition, by oxamniquine, of radiolabelled uridine incorporation into macromolecules in isolated hamster liver nuclei and secondly, to examine the effect of the

oxamniquine analogues, the alcohol (**29**) and the diol (**31**), on uridine incorporation into macromolecules as an indication of their effect on *in vitro* RNA biosynthesis. It was expected that a comparison of the effect of the analogues on *in vitro* RNA biosynthesis with that of oxamniquine would give some indication of the potential schistosomicidal activity of these novel analogues.

It was anticipated that the results obtained in this study would give some indication of the effect of structural modification on biological activity within this series of compounds.

5.2 MATERIALS AND METHODS

5.2.1 Materials

(a) Animals

Male Syrian hamsters, SH4 strain, were used.

(b) Instruments

Homogenizer: Tri-R Stir-R, Model K41, Tri-R Instruments, Rockville Centre New York.

Centrifuges: MSE Bench top centrifuge

Mistral 6L General Purpose Refrigerator Centrifuge, MSE.

LKB Wallac, 1215 RackBeta Liquid Scintillation Counter.

(c) Chemicals

Ribonucleotide triphosphates: uridine 5'-triphosphate (UTP), adenosine 5'-triphosphate (ATP), cytidine 5'-triphosphate (CTP) and guanosine 5'-triphosphate (GTP) were purchased from Sigma Chemical Co.

(2-^{14}C)-Uridine 5'-triphosphate, specific activity 1.92 GBq/mmol, radioactive concentration 1.85 MBq/ml, was a product of Amersham International, Cardiff, U.K.

Dithioreitol and NaHEPES were purchased from Sigma Chemical Co. Analytical grade sucrose was a product of Fisons Fine Chemicals. All other reagents were of analytical grade unless otherwise stated.

(d) Drugs

Oxamniquine, UK 4271 Lot 603/OX/701 was a generous gift from Pfizer Central Research, Sandwich, Kent, England. A 20 mM solution of oxamniquine in 1,4-dioxan was prepared.

The oxamniquine analogues, the alcohol (**29**) and the diol (**31**), were synthesized as described in Sections 2.2.6 and 2.2.7(b) respectively. 20 mM solutions of each compound in 1,4-dioxan were prepared.

(e) Preparation of solutions

(i) Homogenization Buffer (HB)[140]
0.3 M sucrose containing 2 mM $Mg(OAc)_2$, 3 mM $CaCl_2$ and 10 mM Tris-HCl (pH8.0).

(ii) Reaction Mixture (RM)[50]
The reaction mixture was prepared so that a 20 µl aliquot, when diluted to 40 µl in the final incubation mixture, would contain: 25 mM tris-chloride, pH 7.6, 0.25 M sucrose, 0.25 mM $Ca(OAc)_2$, 5 mM $MgCl_2$, 1 mM $MnCl_2$, 0.05 mM EDTA, 2.5 mM dithioreitol, 0.15 M KCl, 0.4 mM of each of ATP, CTP and GTP, 0.05 mM (2-^{14}C) UTP

5.2.2 Methods

(a) Preparation of Nuclei

Hamster liver nuclei were isolated by homogenization and differential centrifugation according to the method of Ernest. [140]

The freshly isolated hamster liver was homogenized, with cooling, in 10ml of the homogenization buffer at one-third full speed for 2 minutes on the homogenizer. The crude homogenate was filtered through 4 layers of cotton gauze and centrifuged at 1000g for 10 minutes. The supernate was aspirated off, the crude nuclear pellet resuspended in 1ml homogenization buffer containing 0.1% Triton X-100 and then homogenized with five strokes of a tight pestle in a Teflon-glass hand held homogenizer. The homogenate was quickly diluted with 4 ml of 2.3 M sucrose to a final concentration of 1.90 M sucrose containing 2 mM $Mg(OAc)_2$, 3 mM $CaCl_2$, 10mM Tris-HCl pH 8.0 and 0.015% Triton X-100. This suspension was layered over 1 ml of a 2.0 M sucrose cushion in the same buffer and centrifuged at 55000g for 60 minutes, at 0°C. The nuclear pellet was carefully drained, rinsed with 3 volumes of tris buffer pH 8.0 and gently resuspended in 0.15 ml of 25% glycerol containing 5 mM $Mg(OAc)_2$, 50 mM NaHEPES (pH 8.0) and 1% bovine serum albumin using a pasteur pipette. The nuclear suspension was quickly frozen in and stored in liquid nitrogen until required.

The average yield of nuclear material was 0.30 g per liver, approximately 4.7% by weight of the wet liver. The number of liver nuclei per unit volume of the nuclear preparation was determined using a haemacytometer. 10 µl of the nuclear preparation was found to contain approximately 10^7 nuclei.

(b) Assay of RNA synthesis

RNA synthesis was measured in a modified RNA polymerase I and II assay mixture as described by Wong[50] with slight modification: (2-^{14}C)UTP was used in place of (5,6-^{3}H)UTP.

The incubation mixture contained in a total volume of 41 µl :- 20 µl of the reaction mixture (RM), 10 µl of the nuclear preparation, 1 µl of (2-^{14}-C) uridine and 10 µl of 1,4-dioxan. The incubation mixture was vortexed to mix and incubated at room temperature for 30 minutes. The reaction was stopped by the addition of 40 µl of cold 20% trichloroacetic acid, the samples vortexed to mix and stored in ice until processed.

Two methods were used to isolate the macromolecule so that the incorporation of radiolabelled uridine could be assayed.

(i) 40 µl aliquots were spotted onto 2.5 cm Whatman No. 3 filter paper discs, washed with 5% cold trichloroacetic acid (10 ml per disc) for at least 10 minutes then rinsed by swirling with 3 volumes of cold 5% trichloroacetic acid followed by swirling with acetone (5 ml acetone per disc) according to the method of Bollum.[141] The filter paper discs were placed on tissue paper, dried in air for 30 minutes then transferred into scintillation vial inserts.

(ii) 40 µl aliquots were also spotted on to Whatman GF/F glass microfibre filter and washed at the pump with 5 % cold trichloroacetic acid (10 ml) followed by acetone (5 ml) and dried in air at room temperature. The filters were then transferred to scintillation vial inserts. This second method was used in an attempt to eliminate the high level of non specific binding observed when the filter paper method was used.

3 ml of the scintillant (Ecosint) was added to each vial, mixed and left to equilibrate over night at room temperature. The level of radiolabelled uridine incorporated into macromolecules, a measure of the RNA polymerase activity of the isolated liver nuclei, was then determined by counting the samples for 10 minutes in the liquid scintillation counter.

The following controls were included in the assay:

(i) Background control: a sample in which the aliquot of radiolabelled uridine was replaced with an equal volume of 'cold' uridine was prepared and treated in an identical manner to the test.

(ii) Control for non specific binding: a sample in which the nuclear preparation was replaced with an equal volume of the storage medium was prepared and treated in an identical manner to the test. This was to correct for any non specific binding of unincorporated radiolabelled uridine to the filter.

The effect of drugs on RNA biosynthesis was investigated by including the drug in the incubation mixture described above. The drugs were initially dissolved in 1,4-dioxan and aliquots were added to the reaction mixture to give final drug concentrations of 5 mM. This concentration was chosen the basis that 5 mM oxamniquine reduced RNA biosynthesis activity in isolated hamster liver nuclei by about 55%.[50]

Two replicate samples were prepared for each assay and all samples were corrected for background radioactivity.

Standard precautions for the handling of ionizing radiations were taken during the assay.

The assay samples were prepared in 150 µl microfuge tubes as shown in Table 5.1.

Table 5.1

Assay samples, volume (μl)				
Component	background control	enzyme blank	no drug	test drug
(^{14}C)-uridine	1 μl UTP	1	1	1
RM	20	20	20	20
dioxan	10	10	10	0
test drug 20 mM	0	0	0	10
nuclear preparation	10	0	10	10
storage medium	0	10	0	0
Total volume (μl)	41	41	41	41

RESULTS

1. The effects of 5 mM oxamniquine on ^{14}C-UTP incorporation into macromolecules is presented in Table 5.2.

Oxamniquine was shown to inhibit the incorporation of radiolabelled uridine. Only 41 % uridine incorporation was observed when the assay was performed in the presence of 5 mM concentration of oxamniquine. A significant amount of non specific binding of the radiolabel to the filter paper was however, observed. The no enzyme assay gave a mean count rate of 488 DPM even after washing with 5 % trichloroacetic acid followed by rinsing with acetone as described by Wong[50] and Bollum.[141]

Table 5.2 Effect of oxamniquine 5mM, on ^{14}C-uridine incorporation

assay	DPM	corrected counts	% UTP incorporation
background	31.4		
	20.5		
	mean 26.0	0	0
no enzyme	556.5		
	419.5		
	mean 488	0	0
no drug	1215.1		
	1049.2		
	mean 1132.2	644.2	100%
5mM oxamniquine	706.2		
	795.7		
	mean 751	263	41%

2. Attempted evaluation of the effects of the alcohol (**29**) and the diol (**31**) on the incorporation of ^{14}C-uridine

The assay was repeated to evaluate the effects of oxamniquine, the alcohol (**29**) and the diol (**31**) on the incorporation of radiolabelled uridine into macromolecules high levels of non specific binding were observed. The results obtained are presented in Table 5.3.

The high levels of non specific binding of the radiolabel to the filter paper precluded the calculation of meaningful values for the rate of uridine incorporation in the presence of the test drugs.

Table 5.3 The effect of oxamniquine, the alcohol (**29**) and the diol (**31**) on incorporation of ^{14}C-uridine

assay	DPM
1µl 14-C uridine + 3 ml ecosint	108670.3
	97776.6
	mean 103223.5
background	21.6
	19.8
	mean 20.7
no enzyme	**1770.4**
	2852.2
	mean **2311.3**
no drug	2916.1
	1731
	mean 2323.6
5 mM oxamniquine	1445.7
	1726.7
	mean1586.2
alcohol (**29**), 5 mM	1186.9
	881.2
	mean 1034.1
diol (**31**), 5 mM	1030.6
	1553.6
	mean 1292.1

DISCUSSION

Oxamniquine, 5 mM solution in dioxan, was observed to inhibit the incorporation of radiolabelled uridine into macromolecules using isolated hamster liver nuclei as the source of RNA polymerase. This inhibition was in the same order of magnitude as that demonstrated by Wong *et.al.*[50] These workers demonstrated 45 % incorporation of uridine into macromolecules in the presence of a 5 mM concentration of oxamniquine. Although this degree of inhibition was demonstrated in the initial experiments the results could not be repeated and attempts to evaluate the effect of the analogues met with little success.

In subsequent assays, as illustrated by the results in Table 5.3, high counts were observed even in those samples which did not have the nuclear preparation. This was thought to be due to the non specific binding of the radiolabelled uridine to the filter paper. It was therefore apparent that the technique used to isolate the macromolecule was inadequate. This technique has been reported to be a rapid and efficient method for the isolation of macromolecules.[141] Inconsistent washing of the filter paper discs may account for the big variation in the counts obtained for replicate samples.

Thus although it was evident that lower counts were obtained in the presence of oxamniquine and its analogues a meaningful interpretation of the results was not possible.

In attempt to eliminate the high level of non specific binding observed in the filter paper disc technique we investigated the use Whatman GF/F glass microfibre filters and filtration under pressure as a means of isolating the macromolecules. This technique was however found to be inadequate and did not retain the macromolecules.

Further work is therefore required to optimise the isolation technique so that non specific binding may be eliminated. The evaluation of the effects of oxamniquine and analogues on RNA biosynthesis in this *in vitro* model may then be possible.

It is important to note that the inhibition of mammalian RNA polymerase by oxamniquine may be a non specific toxic effect. The concentrations required to demonstrate this effect are much higher, in the order of mM, than those attained after therapeutic administration of oxamniquine, in the order of μg/ml. This suggests that the schistosomicidal activity of oxamniquine is not due solely to inhibition of RNA biosynthesis and may be multifactorial.[50]

CONCLUSIONS AND COMMENTS

Conclusion and comments

Several novel analogues of oxamniquine have been synthesized. The 6-hydroxymethyl group of oxamniquine was replaced to yield the following derivatives:- the acid (**9**), the alcohol (**29**), the ketone (**37**), the olefin (**38**), the diol (**31**), the methylthio vinyl derivative (**67**), the methyl vinyl ether (**39**) and the arylamine (**52**).

Some unexpected reactions were observed, for which possible mechanism have been proposed to explain this unusual chemistry and reactivity inherent in the oxamniquine molecule. These include the oxidative cleavage of the methyl vinyl ether (**39**) with perchloric acid to give the aldehyde (**24**) and the synthesis of the methylthio vinyl derivative (**67**) and the diol (**31**) by an intramolecular ylid rearrangement in the reaction of oxamniquine aldehyde (**24**) with dimethyl sulfonium methylide.

Of the analogues synthesized the acid (**9**), the alcohol (**29**) and the diol (**31**) have been screened for biological activity.

The ability of strychnine to potentiate catechol-induced convulsions in the anaesthetized mouse has been demonstrated and it is proposed that this model may be used as an *in vivo* model for detecting convulsant activity. The CNS effects of oxamniquine (**1**), the acid (**9**) and the alcohol (**29**) have been evaluated in this *in vivo* system. The central effects of oxamniquine were not detectable at oxamniquine doses comparable to therapeutic dose levels. However, a CNS depressant effect was observed with doses higher (200 mg/kg) than those used therapeutically. Although the epileptogenic activity of oxamniquine was not demonstrated in the screen, it was confirmed that the depressant activity observed is of central origin. It should be noted that drowsiness is the most commonly encountered side effect of oxamniquine in clinical use. This is in contrast to the convulsant effects of oxamniquine which are of low incidence. The demonstration of the CNS depressant activity was therefore not too surprising.

The alcohol (**29**) was shown to be a more potent depressant than oxamniquine at the same dose level (200 mg/kg) and completely inhibited catechol-induced activity. The acid (**9**) was however, shown to be devoid of any CNS activity in this model.

A preliminary evaluation of the effects of oxamniquine, the alcohol (**29**) and the diol (**31**) on *in vitro* RNA biosynthesis indicated that oxamniquine was able to

inhibit the incorporation of radiolabelled uridine into macromolecules. However, the assay technique was not optimised and the preliminary results obtained with the alcohol (**29**) and the diol (**31**) could not be confirmed.

Further work therefore needs to be done to optimise the technique so that the effect of the alcohol (**29**) and the diol (**31**) on *in vitro* RNA biosynthesis may be evaluated. It is anticipated that this may give some indication of the potential schistosomicidal activity or other biological activity of these compounds and their potential biological applications. The other oxamniquine analogues synthesized also need to be screened for biological activity. Any compounds showing promising activity in this screen would be sent for further evaluation against the schistosome worm to establish whether the compounds have any antischistosomal activity.

The physico-chemical parameters of the analogues, in particular the octanol-water partition coefficient, need to be determined. Knowledge of the partition coefficient would enable the hydrophobicity parameter, Log P, to be calculated. This is a useful prediction of the ability of a drug compound to penetrate the blood brain barrier and the potential CNS effect of the compound. Neutral molecules which penetrate the brain have an optimal Log P value of 2.01. Oxamniquine has an octanol-water Log P value of 2.245 and has been shown to penetrate the brain after intravenous administration to rats. Knowledge of the partition coefficient of the oxamniquine analogues would enable their biological distribution and potential biological activity to be predicted.

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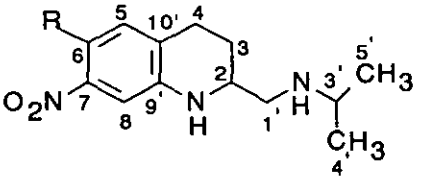
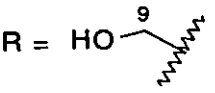
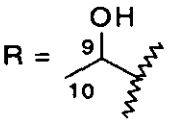
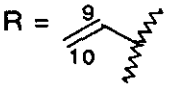
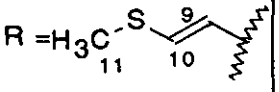
APPENDICES

Appendix I

Selected ^{13}C Chemical Shift Assignments



Oxamniquine (**1**) R = 	δ_C 22.71 (4'-C), 23.24 (5'-C), 25.51 (3-C), 26.33 (4-C), 48.61 (3'-C), 50.99 (2-C), 52.24 (1'-C), 61.86 (9-C), 108.73 (8-C), 124.01 (6-C), 127.49 (7-C), 130.52 (5-C), 144.36 (10'-C) and 146.22 (9'-C).
Alcohol (**29**) R = 	δ_C 22.69 (4'-C), 23.23 (5'-C), 23.82 (10-C), 25.67 (3-C), 26.49 (4-C), 48.74 (3'-C), 51.03 (2-C), 52.31 (1'-C), 64.78 (9-C), 107.98 (8-C), 127.21 (7-C), 127.64 (5-C) and 143.98 (10'-C).
Olefin (**38**) R = 	δ_C 22.76 (4'-C), 23.34 (5'-C), 25.81 (3-C), 26.53 (4-C), 48.97 (3'-C), 51.13 (2-C), 53.34 (2-C), 108.06 (8-C), 114.26 (9-C), 120.94 (10-C), 126.72 (6-C), 128.38 (7-C), 132.64 (5-C), 144.54 (10'-C) and 146.78 (9'-C).
Methylthio vinyl ether (**67**) R = 	δ_C 14.76 (11-C), 22.51 (4'-C), 23.071 (5'-C), 25.79 (3-C), 26.42 (4-C), 49.05 (3'-C), 50.87 (2-C), 52.19 (1'-C), 108.38 (8-C), 119.99 (9-C), 120.75 (6-C), 126.60 (10-C), 127.06 (7-C), 127.94 (5-C) and 143.62 (10'-C).

Appendix II

1. The effect of drugs on catechol-induced activity in the anaesthetized mouse.

(a) Strychnine

(i) Strychnine 0.15 mg/kg i.p.

Protocol:

Catechol 80 mg/kg —90 min→ Drug —30 min→ Catechol 80 mg/kg

Catechol-induced activity prior to test drug =A

Catechol-induced activity after test drug = B

Table 1

assay		duration (min)	% change in duration	number of twitches	% change
M62	A	7.5	30	69	35
	B	11.5		72	
M63	A	12	45	123	67
	B	11.5		153	
M64	A	9	44	160	31
	B	13		209	
M65	A	11.5	33	80	45
	B	11		116	
M66	A	7.7	51	46	116
	B	10		132	
M67	A	12	-8	109	239
	B	16		370	
M68	A	9	44	223	17
	B	12		279	
M69	A	9	-4	195	
	B	12		283	45

(ii) Strychnine 1.5 mg/kg i.p.

Catechol 80 mg/kg —90 min→ Drug —30 min→ Catechol 80 mg/kg

Catechol-induced activity prior to test drug =A

Catechol-induced activity after test drug = B

Table 2

assay		duration (min)	% change in duration	number of twitches	% change
M94	A	16.3	44	155	-26
	B	23.5		115	
M95	A	11.2	-21	55	44
	B	8.9		79	
M100	A	13.5	47	123	12
	B	19.9		138	
M101	A	9.2	74	163	0
	B	16		164	
M102	A	10	-5	175	-47
	B	9.5		92	
M103	A	6.9	29	69	88
	B	8.9		130	
M104	A	11.6	15	44	234
	B	13.3		147	
M105	A	7	66	94	141
	B11.6	227			

(b) Oxamniquine

(i) Oxamniquine 60 mg/kg p.o.

Protocol:

Catechol 80 mg/kg —60 min→ drug —20 min→ Catechol 80 mg/kg

Catechol-induced activity prior to test drug =A

Catechol-induced activity after test drug = B

Table 3

assay		duration (min)	% change in duration	number of twitches	% change
M22	A	17	253	113	50
	B	60		178	
M24	A	15	-100	101	-100
	B	0		0	
M26	A	18	-	48	146
	B	-		118	
M27	A	12	125	199	-67
	B	27		65	
M29	A	21	43	85	152
	B	30		214	
M30	A	18	78	161	35
	B	32		217	
M31	A	13	131	89	95
	B	30		173	

(ii) Oxamniquine 30 mg/kg i.v.

Protocol:

Catechol 80 mg/kg —90 min→ drug —20 min→ Catechol 80 mg/kg

Catechol-induced activity prior to test drug =A

Catechol-induced activity after test drug = B

Table 4

assay		duration (min)	% change in duration	number of twitches	% change
M37	A	8	106	174	55
	B	16.5		270	
M40	A	23	9	192	194
	B	25		565	
M42	A	8	19	178	-1
	B	9.5		180	
M44	A	7	-100	49	33
	B	11.8		65	
M45	A	16	50	224	19
	B	24		266	
M46	A	22	11	203	16
	B	24.5		236	
M48	A	10	100	132	-36
	B	20		85	
M50	A	7	14	73	-78
	B	6		16	

(iii) Oxamniquine 200 mg/kg i.p.

Protocol:

Catechol 80 mg/kg —90 min→ drug —60 min→ Catechol 80 mg/kg

Catechol-induced activity prior to test drug =A

Catechol-induced activity after test drug = B

Table 5

assay		duration (min)	% change in duration	number of twitches	% change
M56	A	13.5	-100	6.5	-100
	B	0		0	
M57	A	10.5	-100	28	-100
	B	0		0	
M58	A	9	17	144	-85
	B	7.5		22	
M59	A	6.5	-100	94	-100
	B	0		0	
M60	A	8.3	-100	85	-100
	B	0		0	
M61	A	9.5	37	170	-56
	B	13		75	
M87	A	8.5	-100	135	-100
	B	0		0	

(c) The alcohol (29) (200 mg/kg i.p.)

Protocol:

Catechol-induced activity prior to test drug =A

Catechol-induced activity after test drug = B

Table 6

assay		duration (min)	% change in duration	number of twitches	% change
M74	A	17	-100	219	-100
	B	0		0	
M75	A	5	-100	47	-100
	B	0		0	
M76	A	10.5	-100	79	-100
	B	0		0	
M79	A	7.75	94	155	38
	B	15		245	
M92	A	7.5	-100	77	-100
	B	0		0	

(d) The acid (9) (140 mg/kg i.p.)

Protocol:

Catechol 80 mg/kg —90 min→ drug —60 min→ Catechol 80 mg/kg

Catechol-induced activity prior to test drug =A

Catechol-induced activity after test drug = B

Table 7

assay		duration (min)	% change in duration	number of twitches	% change
M80	A	4	100	24	283
	B	8		92	
M81	A	15	27	196	27
	B	19		248	
M82	A	11	50	99	93
	B	16.5		191	
M83	A	19	16	218	33
	B	22		291	
M84	A	7	61	36	622
	B	18		260	
M86	A	9.5	-12	38	216
	B	8.5		120	
M89	A	14	93	29	1003
	B	27		320	
M91	A	18	6	161	41
	B	19		227	

2. The effect of drug vehicle on catechol-induced activity

(a) Saline (0.4ml i.p.)

Protocol:

Catechol 80 mg/kg —(90 min)→ drug vehicle —(30 min)→ Catechol 80 mg/kg

Catechol-induced activity prior to drug vehicle =A

Catechol-induced activity after drug vehicle = B

Table 8

assay		duration (min)	% change in duration	number of twitches	% change
M68	A	9	25	223	25
	B	12		279	
M69	A	9	25	195	25
	B	9		243	
M70	A	10	-20	211	-20
	B	11.5		164	
M71	A	8	3	202	3
	B	10		208	
M73	A	13.5	73	104	73
	B	20		180	

(b) Effect of 1% Tween 80 (1 ml p.o.)

Protocol:

Catechol 80 mg/kg —60 min→ drug vehicle —20 min→ Catechol 80 mg/kg

Catechol-induced activity prior to drug vehicle =A

Catechol-induced activity after drug vehicle = B

Table 9

assay		duration (min)	% change in duration	number of twitches	% change
M32	A	17	41	21	638
	B	24		155	
M33	A	20	-100	135	-100
	B	0		0	
M34	A	11.5	30	67	16.4
	B	15		78	

(c) Citrate-phosphate buffer pH 5.0 (i.v.)

Protocol:

Catechol 80 mg/kg —60 min→ drug vehicle —20 min→ Catechol 80 mg/kg

Catechol-induced activity prior to drug vehicle =A

Catechol-induced activity after drug vehicle = B

Table 10

assay		duration (min)	% change in duration	number of twitches	% change
M37	A	8	106	174	55
	B	16.5		270	
M39	A	3	400	21	93
	B	15		308	
M41	A	8.4	135	194	70
	B	20		330	
M43	A	10	45	102	3
	B	14.5		107	
M45	A	11	45	222	1
	B	16		224	
M47	A	5	0	44	43
	B	5		63	
M49	A	6.5	0	47	-66
	B	6.5		16	

(d) Citrate-phosphate buffer pH5.0 (0.4 ml i.p)

Protocol:

Catechol 80 mg/kg —60 min→ drug vehicle —60 min→ Catechol 80 mg/kg

Catechol-induced activity prior to drug vehicle =A

Catechol-induced activity after drug vehicle = B

Table 11

assay		duration (min)	% change in duration	number of twitches	% change
M94	A	16.3	4.5	155	-26
	B	23.5		115	
M95	A	11.2	-20.4	55	44
	B	8.85		79	
M100	A	13.5	47.4	123	12
	B	19.9		138	
M101	A	9.2	73.9	163	0
	B	16		164	
M102	A	10	-5	175	-4.7
	B	9.5		92	
M103	A	6.9	29	69	88
	B	8.9		130	
M104	A	11.6	14.7	44	234
	B	13.3		147	
M105	A	7	65.7	94	141
	B	11.6		227	