SYNTHESIS AND REACTIONS OF CYCLOPROPAMITOSENES AND RELATED PYRROLO[1,2-a]INDOLES

by

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A Doctoral Thesis

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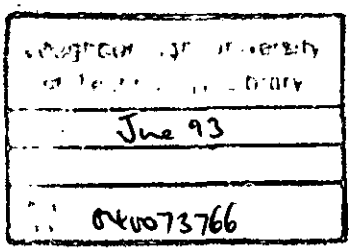

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Abstract:

The use of Mitomycin C in the treatment of a wide range of neoplastic conditions is discussed. The mechanism of action of mitomycin anticancer antibiotics under reductive activation conditions using either enzymes, sodium dithionite, catalytic hydrogenation or chromium (II) perchlorate is examined, as is the alkylation of DNA.

An intramolecular [3+2] cycloaddition strategy has been employed to synthesise the pyrrolo[1,2-a]indole nucleus for a wide range of cyclopropamitosenes, whereas the key step in the synthesis of pyrrolo[1,2-a]indoles without the cyclopropane ring was a modified Wittig reaction.

From the onset of the work it was important to investigate the role of the 7-methoxy group. Hence a variety of cyclopropamitosenes or related pyrrolo[1,2-a]indoles were subjected to C-7 exchange reactions with either (i) other alkoxides or (ii) cyclic / acyclic amines. In this way, structural modification at C-7 can be related to the biological results.

Biological and electrochemical data were recorded for the cyclopropamitosenes and related pyrrolo[1,2-a]indoles and correlated with their structures.

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Finally, I would like to thank my parents and sisters for their continued support throughout my education.

TO MY PARENTS, CRAZY SISTERS,

AND IN THE MEMORY OF MOLLY O' SULLIVAN AND MICHAEL O' CONNOR
(MAY THEY REST IN PEACE)

WITH ALL MY LOVE

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Abbrevitaions

Ac	Acetyl
Bn	Benzyl
DCM	Dichloromethane
DMF	*N,N*-Dimethylformamide
DMSO	Dimethyl sulphide
DNA	Deoxyribonucleic acid [Appendix 1]
EDC	Ethylene dichloride
FAB	Fast Atom Bombardment
FAD	Flavin adenine dinucleotide
ESR	Electron Spin Resonance
HPLC	High performance liquid chromatography
IR	Infra-red
i.v.	Intravenous administration
MMA	Mitomycin A
MMC	Mitomycin C
m.p.	Melting point
NAD^+, NADH	Nicotinamide adenine dinucleotide and its reduced form
$NADP^+$, NADPH	Nicotinamide adenine dinucleotide phosphate and its reduced form
3NBA	3-Nitrobenzyl alcohol
NMFA	*N*-Methylformanilide
NMR	Nuclear Magnetic Resonance
nOe	Nuclear Overhauser Enhancement
py	Pyridine
r.t.	Room temperature
SM	Starting material
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMS	Trimethylsilyl
Tos	Toluenesulphonyl
V_{max}	The maximum velocity of an enzymatic reaction (Appendix 2)
K_M	Michaelis constant (Appendix 2)

Note: (i) MeO Series refers to the synthetic route for the preparation of 7-methoxycyclopropamitosene (57).

(ii) MeO-Me_2 Series refers to the synthetic route for the preparation of 1a,1a,-dimethyl-7-methoxycyclopropamitosene (58)

CHAPTER 1

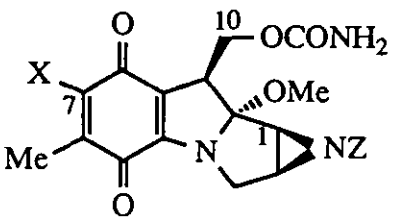
THE CHEMISTRY AND BIOLOGY OF THE MITOMYCIN ANTITUMOUR ANTIBIOTICS AND BIOREDUCTIVE DRUGS

1.1. Introduction To The Mitomycin Antitumour Agents

Mitomycins were first discovered in 1956 by workers at Kyowa Hakko Kogyo Co., a Tokyo drug company, among fermentation products of *Steptomyces caespitosus*. A Japanese group led by T. Hata in Kitasato Institute isolated mitomycins A and B from a species of actinomyces named *Steptomyces caespitosus.*[1] Extraction of the fermentation broth with chloroform and chromatography of the crude extracts on alumina gave two compounds which were recrystallised from acetone as crystalline solids, designated mitomycin A[2] and mitomycin B.[3] Two years later, workers in Kyowa Fermentation plant, attempting production of mitomycins A and B, noticed that at slightly higher pH there was diminished recovery of mitomycins A and B in favour of a new compound, mitomycin C (MMC) (1),[4] which is an antibiotic with antitumour activity.[5, 6]

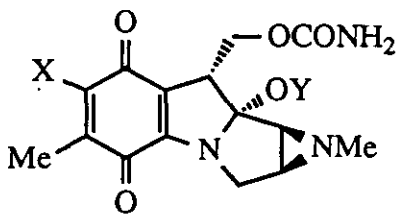
Over the last thirty years or so, developments in isolation and structural elucidation techniques have led to the discovery of a large number of closely related structures, most of which compounds demonstrate some antitumour and also antibacterial activity.[7] Hence the mitomycins have been divided into three main groups based on similarities in chemical structures. These are designated groups A, B, and G so called after the first member of each type to be isolated, as illustrated below.

A Type



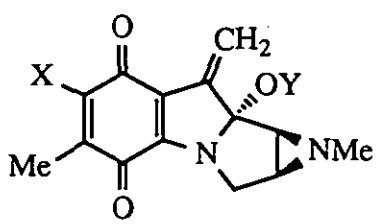
X	Z	Name
MeO	H	Mitomycin A
NH_2	H	Mitomycin C (1)
MeO	Me	Mitomycin F
MeNH	H	Mitomycin M
NH_2	Me	Porfiromycin

B-Type



X	Y	Name
MeO	H	Mitomycin B
NH_2	H	Mitomycin D
MeO	Me	Mitomycin J
MeNH	H	Mitomycin L

G Type



X	Y	Name
NH_2	Me	Mitomycin G
MeO	Me	Mitomycin K
MeO	H	Mitomycin H

All members of the mitomycins in groups A, B, and G are characterised by the azirino[2',3':3,4]pyrrolo[1,2-a]indole ring system. Mitomycins in groups A and B also have a peculiar chemical structure, in so far as they possess three potentially cytotoxic groups namely (i) aziridine ring, (ii) carbamate, and (iii) quinone on the same pyrrolo[1,2-a]indole nucleus. There is some confusion about the nomenclature and numbering of the mitomycin structures. In the initial structure elucidation of the mitomycins, the numbering is illustrated below in *Figure 1*. However, *Chemical Abstracts* uses a highly systematic name and a different numbering system.

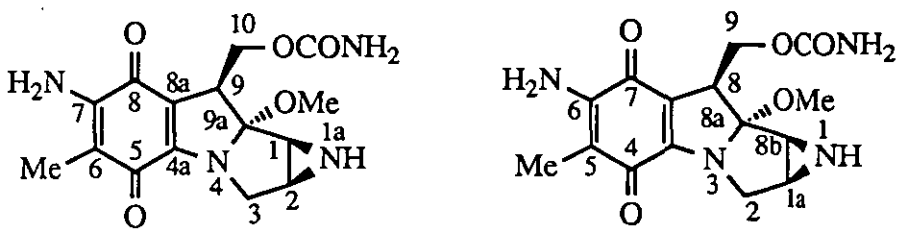


Figure 1: *MMC numbering systems. Top left, mitosane: top right Chemical Abstracts system*

Thus, the trivial name of MMC is 7-amino-9a-methoxymitosane, but it is known in *Chemical Abstracts* as 6-amino-1,1a,2,8,8a,8b-hexahydro-8-(hydroxymethyl)-8a-methoxy-5-methyl-azirino[2',3':3,4]pyrrolo[1,2-a]indole-4,7-dione carbamate.

Commercial production of large batches of MMC by fermentation of *Steptomyces caespitosus* has allowed its development as a clinical anticancer agent. MMC is an antitumour antibiotic that is activated in tissues to either a mono or bisfunctional alkylating agent. It disrupts DNA in cancer cells by forming a complex with DNA. This inhibits division of cancer cells by interfering with the biosynthesis of DNA, probably at the DNA replication stage.[8] The use of MMC as an antitumour drug was first developed in Japan in the early sixities.[9] Europe and the United States did not follow the pace set by Japan as far as the use of this drug was concerned, and MMC usage has been slow to

develop. In the United States it was officially introduced for clinical practice in 1974.[9] However, since then the clinical use of this drug has acquired growing importance. MMC is recommended for certain types of cancers, either alone or in combination with other drugs. One of MMC peculiar's features is its degree of selectively based on the reductive activation to cytotoxic metabolites in hypoxic cells. These hypoxic cells are the main limitation to the use of radiotherapy and this is the rationale for using MMC in combination with radiotherapy. MMC in particular, has been successfully used in the treatment of a wide range of neoplastic conditions including carcinomas (epithelial cells) of the stomach, pancreas, lung, colon, breast, bladder and uterus.[10] In addition MMC has been of some use in the treatment of acute and chronic leukaemias and Hodgkin's disease. Despite its broad spectrum of activity, MMC suffers from the fact that extended use can lead to severe myelosuppression (bone marrow suppression) which can be fatal. From the onset of the work on this project one of our objectives was to synthesise a large number of MMC analogues and examine them *in vitro* and *in vivo* for antitumour activity with the purpose of enhancing antitumour potency and to reduce myelosuppression. This review gives a brief insight into the clinical usage of MMC at the present time, and also attempts to highlight the apparent mode of action of mitomycins *in vivo*. Finally, a brief consideration into the design and use of alternative bioreductive drugs is discussed.

1.2. Clinical Usage of MMC

1.2.1. Activity in Experimental Model Animals

MMC has a wide range of antitumour activity in the treatment of leukaemias, lymphoma, sarcomas and melanoma in experimental laboratory animals such as mice, rats, hamster, and chickens. Often several standard solid tumours and leukaemia lines are used for comparison against other anticancer agents, such cell lines are; L-1210 and P-388 murine leukaemia systems, Walkers carcinosarcoma system, and the Dunning system.[6] This is shown below in *Table 1*, which compares the effectiveness of MMC to a variety of well known antitumour agents on various solid tumours and leukaemia cell lines.

It should be noted that the higher the value the more effective is the anticancer agent. From *Table 1* it is shown that MMC has the widest general effectiveness across all the four groups, Walker carcinosarcoma as well as leukaemias such as P-388.

Table 1: Comparison of Antitumour Activity for 9 Clinically Anticancer Drugs

	P-388	L-1210	Walker	Dunning
Mitomycin C	75	27	97	215
Fluorouracil	60	40	73	96
Methotrexate	61	67	97	109
Daunomycin	64	39	92	No data
Cytarabine	61	63	33	34
Vinblastine	56	27	91	100
Vincristine	68	26	82	104
Cyclophoshamide	69	53	92	250
Melphalan	100	50	97	200

ILS: Increased life scan, for L-1210 (150%), P-388 (200%), Dunning (80%); TWI: Tumour weight index = 98%.

1.2.2. Clinical Antitumour Activity

The initial clinical trials for MMC were done in Japan and reviewed by Frank and Osterberg.[10] From the initial results base on a wide variety response criteria, they concluded that MMC gave an overall 37% success rate for patients suffering from advanced solid tumours. In the United States, however, initial trials were less promising, due to high clinical toxicities encountered. Hence, when the toxicities were controlled by using finely controlled dosage schedules, the success rate went up to 30% for patients with advanced epithelial tumours.[11] In a later study, a good activity response rate of 17.6% (17.6% of 85 patients) was observed, using MMC in patients with advanced gastrointestinal cancers. This result is comparable with 5-fluorouracil (17% of 290 patients) which is the best current single agent in this category.[12] As already stated, MMC also shows selective activity against hypoxic cells.

1.2.3. Clinical Results of Using MMC Alone or In Combination Chemotherapy

More than a quarter of a million people develop cancer each year in the United Kingdom.[13] Based on National incidence rates, an estimated one in three will develop cancer during their lifetime.[13] However, it is likely to be later in their life, as over 70% of all new cancers develop in people aged 60 years or more.[13] By far the commonest

cancer in men is lung cancer, responsible for nearly a quarter of all new cases in men. For women, the commonest is breast cancer (19%). Skin cancer, excluding melanoma, is the second commonest cancer for both men and women: fortunately this type of cancer is nearly always curable. In 1989 over 163,000 people died from cancer in the United Kingdom. Cancer is now the cause of a quarter of all deaths and a quarter of all cancer deaths are caused by lung cancer.[13]

The main effectiveness of MMC either alone or in combination in the treatment of lung, breast, head, neck, cervical and stomach cancer will be considered. These are some of the important observations from clinical trials done in Italy prior to 1991.

Breast Cancer

Breast cancer is the main cause of death amongst women especially in Europe and North America. In the United Kingdom in 1989, 19% of women developed cancer, and this this cancer is responsible for a fifth of all female cancer deaths.[13] MMC is usually a second or third choice drug. With advanced breast cancer, a 26% response rate has been recorded, using MMC alone.[14a] MMC has been used as the following combinations in the treatment of this cancer; (i) adriamycin + MMC; (ii) fluorouracil + adriamycin + MMC + vincristine; (iii) MMC + methotrexate + mitroxantrone;[14a] (iv) MMC + etoposide + fluorouracil + cisplatin, the later giving a high rate of objective responses of 67% in 15 patients.[14b]

Cervical Cancer

In 1989 in The United Kingdom death due cervical cancer accounted for 3% of all female tumour death.[13] Combination chemotherapy as a first therapeutic step in the treatment of cervical cancer has produced a number of good responses in the advanced stages. Thomas *et al.* have reported a high percentage of complete responses following the chemo-radiotherapic approach using MMC + fluorouracil + radiotherapy in patients.[15]

Lung Cancer

Lung carcinomas accounted for 32% (27,240 men) of all male tumour-related deaths and for 16% (12,410 women) of all female tumour related deaths in the United Kingdom in 1989.[13] Chemotherapy appears more effective when administered in the early stages of this disease. Using single agent chemotherapy gives only 15-20% (MMC = 20%) positive responses in non-small cell carcinonomas and 20-40% in the small cell type.[16] The best results are obtained using combined chemotherapy, often containing MMC. Response rates vary between 30-56%.[16] In one reported study by Chang *et al.*[16] a

combination therapy of (i) MMC, 20 mg / m^2, (i.v.) day 1; + (ii) cisplatin 50 mg / m^2, (i.v.) days 1, 22; and (iii) vincristine 1.2 mg / m^2, (i.v.) day 1-every six weeks is active against all histotypes reaching 47% objective responses in patients who had not previously received radiotherapy, and 33% in pretreated patients. The interesting point here is that large cell carcinoma subtype, which is usually less responsive to chemotherapy, appears quite responsive.
Note: mg / m^2 of body suface area

Gastric Cancer

Gastro-intestinal carcinomas are amongst the most frequently encountered tumours and among the main causes of tumour-related deaths. In 1989 deaths due to cancer of the stomach accounted for 7% of all male and 5% of all female deaths in the United Kingdom.[13] The effectiveness of MMC as a single agent chemotherapy in gastric cancer is good having a high objective responses of 24% [17] (498 patients were used in the study). A chemotherapy technique developed by Mac Donald *et al.* in the treatment of advanced carcinoma of the stomach uses a combination of (i) fluorouracil 600 mg / m^2, (i.v.) days 1, 8, 29, 36; + (ii) adriamycin 30 mg / m^2 (i.v.), days 1, 29; and (iii) MMC 10 mg / m^2 (i.v.), day 1-every eight weeks produced a 35% objective responses.[17]

Head and Neck Cancers

Japanese workers reported a 22% response rate for these cancers, using MMC as a sole agent.[18]

In Summary: MMC is clinically very useful against a wide variety of solid tumours. This drug has activity against solid tumours that are not sensitive to others agents. It is probably the single most active agent against non-small cell lung cancer and amongst the best against gastrointestinal and breast cancer. MMC shows some selectivity for hypoxia cells. It would be of importance to us in the West to synthesise analogues which enhance the antitumour activity but diminish the side effects and the search for these has intensified in the last couple of years.

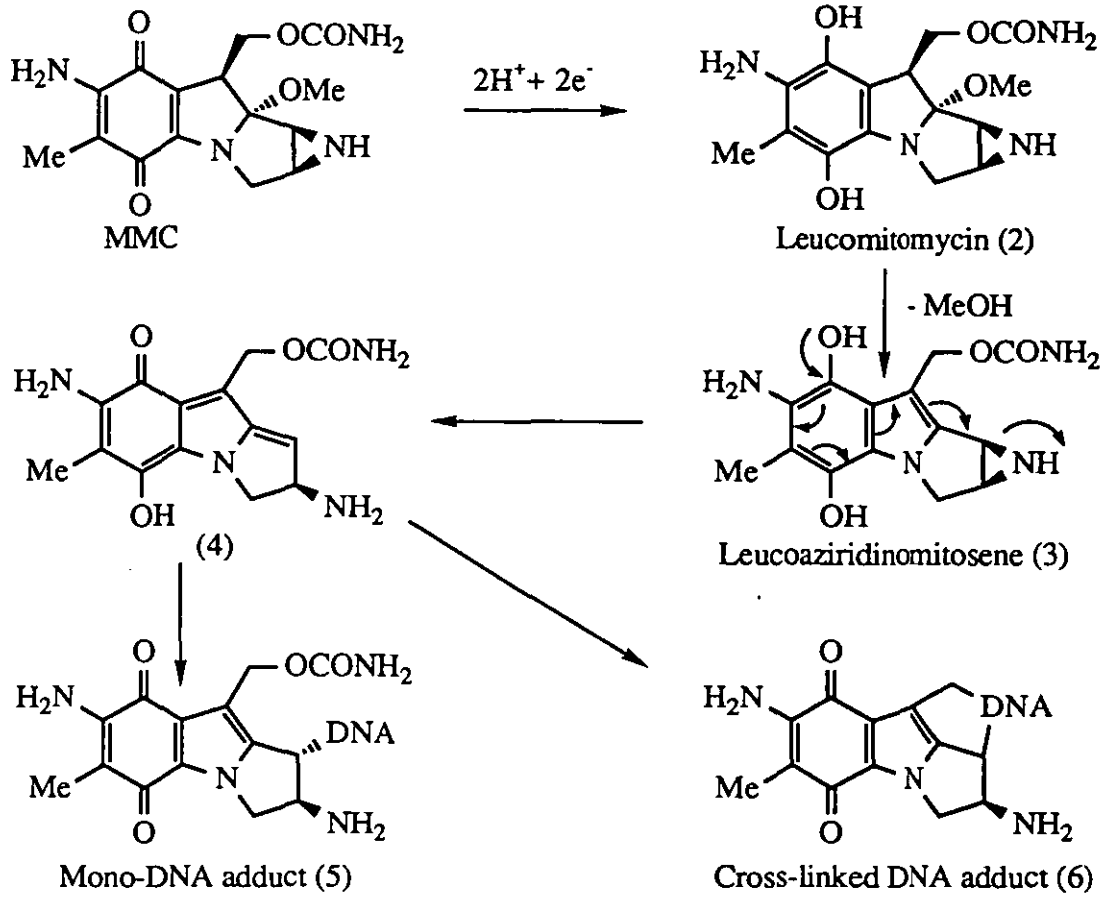
1.3. The Mode of Action of the Mitomycins Particularly MMC In Vivo

MMC, the potent antibiotic and clinically useful antitumour agent binds covalently to DNA both in a monofunctional and bifunctional manner, resulting in the latter case, in stable cross-links between complementary strands both *in vitro* and *in vivo*.[5, 19] The proportion of cross-linked DNA is only about 10% of mono alkylation,[20] suggesting that

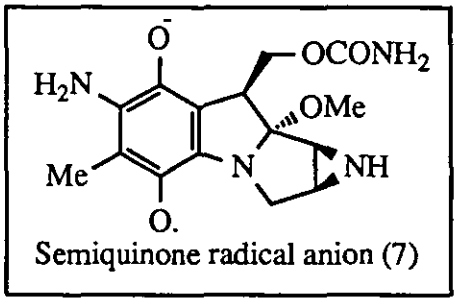
there might be a minor pathway with different factors controlling the products outcome. Both mono and bis DNA adducts can prevent DNA replication in tumour cells, but it should be noted that radical reactions which generate superoxide radicals can also cause DNA strand scission and could contribute to some of the observed antitumour activity.[21] Both the mono and bis alkylation processes require activation of MMC by reduction of the quinone system.[19] This is supported by the observation that unreduced MMC cannot bind to nucleophiles such as (i) phosphates supplied by inorganic phosphates, (ii) 2'-deoxyguanosine, (iii) d(GpC) [Appendix 1], (iv) guanosine residues and (v) DNA unless a pH of 4 is obtained.[22, 23, 24, 25] This is unlikely in most tissues, although not entirely impossible in some acidic tumour cells. *In vivo* this occurs by the action of flavoreductases such as DT-diaphorase, and NADPH : cytochrome P450 reductase which catalyse the conversion of MMC into its cytotoxic metabolites.[26] The *in vivo* conditions can be mimicked by either mild acid treatment or under reductive activation conditions using enzymatic,[27, 28, 29, 30] chemical,[30, 31, 32], catalytic hydrogenation,[27, 33, 34] and electrochemical methods,[35] at physiological pH. Thus, MMC represents an example of the class of substances that function as *bioreductive alkylating agents*.

The first covalent binding to DNA with MMC *in vitro* was achieved by Szybalski and Iyer [19] in 1964 and the authors postulated the generally accepted mode of action of MMC. Iyer *et al.* observed that mitomycins and porofiromycin behaved as bifunctional alkylating agent upon chemical reduction with sodium dithionite, catalytic hydrogenation using 5% palladium on carbon, or enzymatic reduction with *Sarcina lutea* DNA. A high content of guanine and cytosine favoured this cross-linking reaction. From these observations, it was concluded that the C-1 aziridine and the C-10 carbamate groups were two masked alkylating functions, which become "allylic" under reductive activation conditions, affording the hydroquinone (2) as shown in *Scheme 1*. Consequent spontaneous elimination of methanol from the 9a position to affords (3). In this hypothesis the driving force for the elimination process would be release of the steric strain energy of the aziridine ring during the formation of (4). This species (4) can then react further to give the mono-DNA adduct (5) or cross-linked DNA (6). It took many years to provide the experimental evidence to confirm this postulated mechanism. Moore amended this hypothesis by speculating that both displacement were S_N1 types taking place sequentially.[36]

Scheme 1: The Moore (Iyer-Szybalski) Mechanism for the Mode of Action of MMC



However, the situation is a little more complicated than it first appears. Iyer-Szybalski and Moore suggested the hydroquinone (2) was the reactive species, but recently it has been postulated that a semiquinone radical anion (7) is the reactive species. It has also been suggested that MMC may go through either species, depending on the situation.

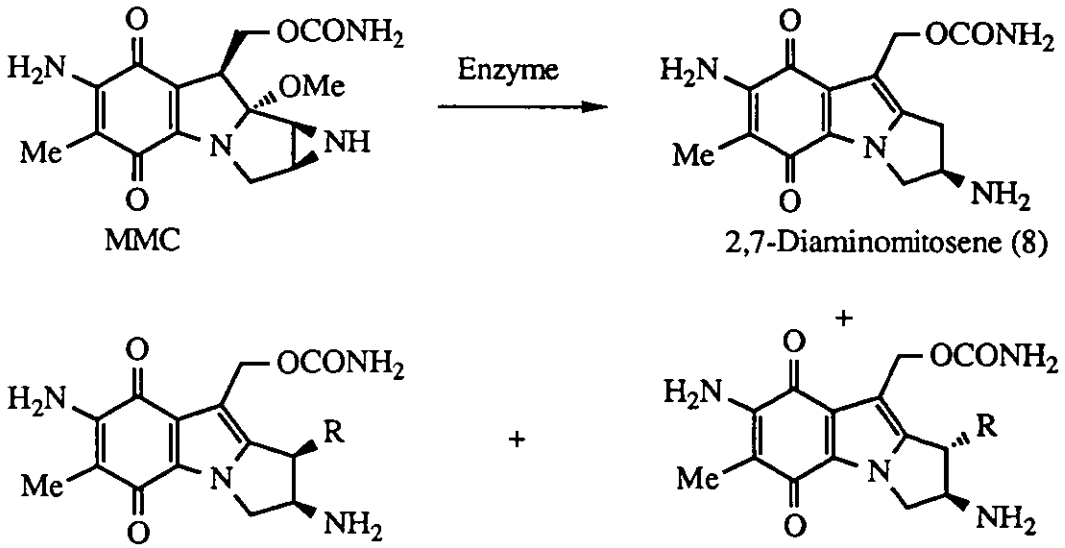


The postulate that the initial binding to DNA is via the semiquinone radical anion (7) is supported by the following experimental observations. Firstly, Tomasz *et al.* in 1974 solved the general problem of low binding *in vitro* by adding sodium dithionite in five equal portions at 5 minute intervals to a solution of polynucleotide and MMC in a sodium

phosphate buffer (pH 7.5) under anaerobic conditions.[37] Using this technique, resulted in approximately 5 and 2-3 binding ratios in native and denatured DNA respectively, which was a vast enhancement. During the course of the reaction, MMC was kept in excess but with a high semiquinone radical anion (7) concentration. This does not confirm that the intermediate is the semiquinone radical anion (7) *in vivo*, but it is an indication. This result is in contrast with Iyer-Szybalski when they only achieved incorporation of 1 unit of MMC per 150 nucleotides with native DNA and 50 nucleotides with denaturated DNA.

Secondly, Pan *et al.* investigated the reductive activation of MMC catalysed by purified microsomal NADPH : cytochrome P450 reductase and bovine milk xanthine oxidase.[28] The reaction was done in a mixture of 0.1 M phosphate buffer and DMSO at pH 7.8 (xanthine oxidase) and pH 7.4 (NADPH : cytochrome P450 reductase), and the metabolites were isolated by sensitive reversed phase HPLC, as shown in *Scheme 2*.

Scheme 2: *Metabolites of MMC after Metabolism by NADPH : cytochrome P450 reductase or xanthine oxidase*

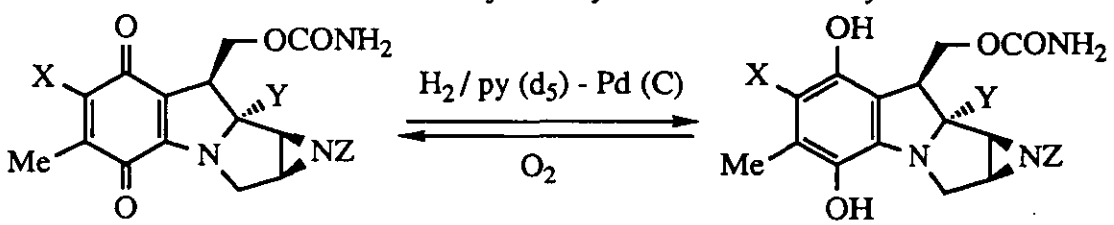


This group proposed that the critical step is a one electron reduction shown by the fact that NADPH : cytochrome P450 reductase and xanthine oxidase favour 1-electron transfer to suitable receptors. MMC thus receives one electron to form a semiquinone radical anion (7) which then undergoes subsequent conversion to metabolites. This is also in agreement with recent results that MMC is a very poor substrate, maybe even an inhibitor of DT-diaphorase.[38] Pan *et al.*, obtained EPR (Electron Paramagnetic

Resonance) evidence which also confirmed this concept.

Thirdly, evidence from Danishefsky and coworkers. Prior to the work done by Danisheksky it was assumed that upon reduction of the quinone to the hydroquinone level, the C-9a hetero function was lost, resulting in the formation of the indolohydroquinone. However, this was not the case as shown in *Scheme 3*, where the mitomycins (MMC, mitomycin B, porofiromycin, and *N*-methylmitomycin A (13), some of which as shown below) are reduced to the leucomitomycins (14), over palladium-charcoal in pyridine and then reoxidised to the mitomycins quantitatively in air.[39, 40]

Scheme 3: Reduction of Mitomycins to Leucomitomycins



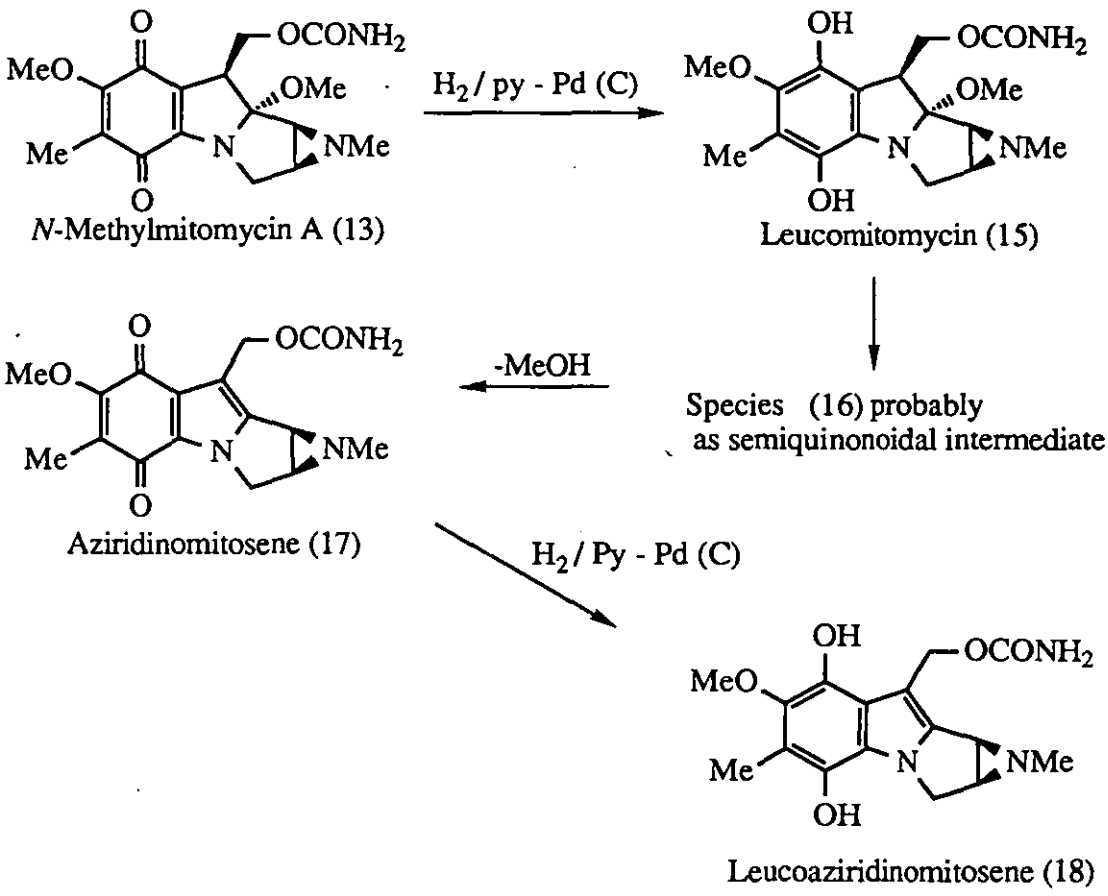
Leucomitomycin (14)

MMC: X= NH_2, Y = OMe, Z = H

N-Methylmitomycin A (13): X = OMe, Y = OMe, Z = Me

The authors reasoned from this experiment that the hydroquinone was not the active species that immediately eliminated the C-9a hetero functions but rather that this was done by another intermediate, maybe a semiquinonoid intermediate. This idea was confirmed by an experiment that is illustrated in *Scheme 4*.[40] Egbertson and Danisheksky mixed a 1:1 solution of the quinone (13) and the hydroquinone (15), and after evaporating the mixture to dryness in the absence of oxygen a 1:1 mixture of mitomycin (15) and the mitosene (17) was isolated as shown in *Scheme 4*. Evaporation and redissolution of a 1:1 solution of (13) and (15) in the presence of external oxygen produced mainly aziridinomitosene (17) and traces of (13) and (15). These experiments concluded that the leucomitomycins are not the reactive intermediates that eliminate the C-9a-hetero function, thus justifying the probability of a semiquinone radical anion species. The aziridinomitosene (17) was reduced to the leucoaziridinomitosene (18) as discussed and for the first time a solution of unstable leucoaziridinomitosene was isolated.

Scheme 4



N-Methylmitomycin A (13)

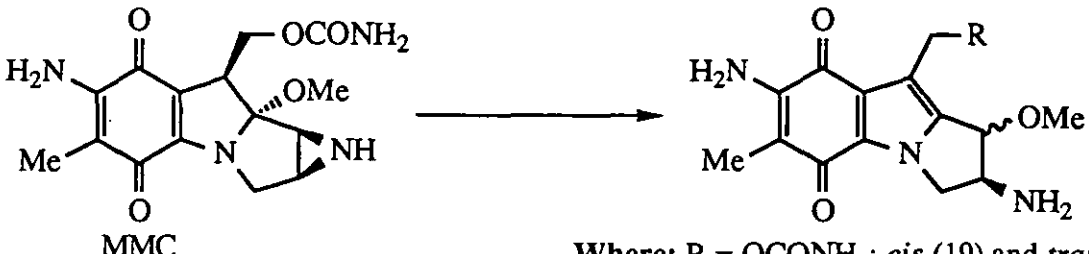
Leucomitomycin (15)

Aziridinomitosene (17)

Leucoaziridinomitosene (18)

Further evidence was supplied by Kohn and coworkers.[35] Kohn's work provided evidence of a 1-electron reduction of the quinone ring of MMC in the polar protic solvent methanol, which led to the loss of methanol followed by ring opening of the aziridine moiety. It was observed by cyclic voltammetry that a one electron transfer process at -0.8 V occurred and a second at -1.5 V, when using a mercury electrode and methanol as the solvent and nucleophile. The observed products profiles were dependent upon the solvent and applied potential as illustrated in *Scheme 5*.

Scheme 5



MMC

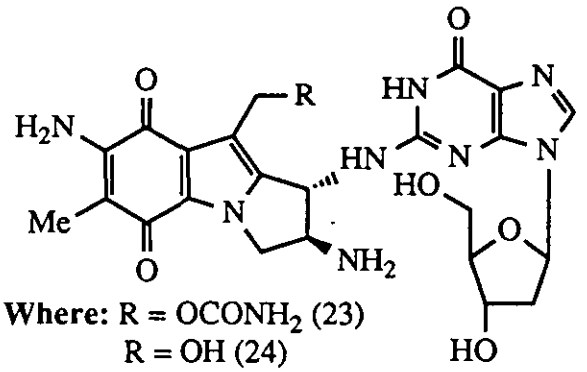
Where: R = $OCONH_2$; *cis* (19) and *trans* (20)
R = H; *cis* (21) and *trans* (22)

Applied Potential = -0.8 V (Hg electrode) led to 1:1 mixture of (19) and (20)
= -1.5 V (Hg electrode) initially led to a 1:1 mixture of (19) and (20)

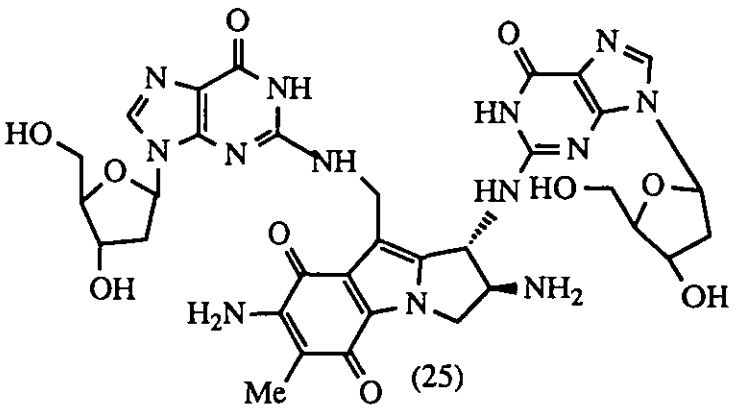
but continued electrolysis led to a decrease of these and increase of (21) and (22), which was independent of pH.

It was also demonstrated by ESR spectroscopy that a one electron addition gave a strong free radical signal for g = 2.01. All this evidence suggests a semiquinone radical anion (7) species but still there is controversy over the exact reactive intermediate involved. Perhaps both the hydroquinone (2) and semiquinone radical anion (7) are reactive intermediates that are expressed at different times under various conditions.

The experimental evidence that MMC was a bisalkylating agent which was first postulated by Iyer, came from work by Tomasz and Nakanishi in 1987.[30] Their group had been involved *in vitro* investigations of the binding of MMC with DNA, in the hope of being able to isolate and fully characterised a bis C-1 and C-10 adduct. This was achieved by exposing MMC to *Micrococcus luteus* DNA in neutral buffer at room temperature under reductive conditions, the complex was digested by a mixture of deoxyribonuclease I, snake venom diesterase, and alkaline phosphatase. The digest was analyzed by HPLC, which enabled them to fully characterise both a mono C-1 (23) and bis C-1+C-10 adduct (25). Also isolated was the decarbamoyl adduct (24) which resulted from nucleophilic attack of water at the iminium intermediate linked through C-1. All three are linked through the N-2 position of 2'-deoxyguanosine.



Where: R = $OCONH_2$ (23)
R = OH (24)

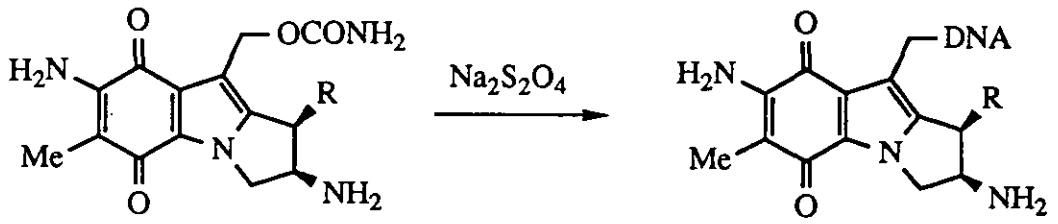


(25)

What was important in their experiments were the striking variations in adduct distribution under different reductive activation conditions. When the authors used catalytic hydrogenation (hydrogen / platinum oxide) conditions, they obtained 90% of the C-1 mono adduct (23) and a trace of (24). However, when switching to sodium dithionite as the reducing agent, no mono adduct was detected, instead the predominant adducts were bifunctional alkylation products (24) and (25) in approximately equal amounts. Also surprisingly, when poly (dGdC)-[Appendix 1] was treated with MMC and activated with sodium dithionite, the bis-DNA adduct (25) was the sole product of alkylation. The authors in an attempt to rationalise these observations, attributed the findings to the better reducing ability of sodium dithionite. They claimed that when using sodium dithionite as the reductant, they got complete reduction. The MMC system was always at the semiquinone level, thus once the mono link between C-1 and dG (deoxyguanosine-5'-monophosphate, a nucleotide) was formed, it was still in the semiquinone state therefore elimination of the carbamate occurred. The resulting iminium species can be trapped with water to afford (24) or dG (deoxyguanosine-5'-monophosphate, a nucleotide) to yield (25). However, under hydrogenation conditions, the semiquinone C-1 bound adduct is oxidised to the quinone level, thus preventing expulsion of the carbamate and subsequent bis adduct formation.

This idea that sodium dithionite is a superior reducing agent over catalytic hydrogenation for mono and bis alkylation of DNA is supported by Iyengar, Dorr, Shipp, and Remers in a recent paper.[41] In their work, they showed that it was possible to monalkylate the C-10 position of either 2,7-diaminomitosene (8) or *cis*-2,7-diamino-1-hydroxymitosene (9) in the presence of calf thymus DNA, resulting in alkylation of the DNA to the extent of 1 molecule per 14 and 11 bases, respectively, as shown in *Scheme 6*. However, no covalent binding was observed on catalytic reduction. The authors rationalised these results as differences in mechanism. Catalytic reduction is a 2-election process that gives a relatively stable hydroquinone, whereas dithionite reduction is a 1-electron process that gives an unstable radical anion. The resulting anion readily loses the carbamoyloxy substituent to afford species that alkylate at the C-10 position.

Scheme 6



Where: R = H (8)
R = OH (9)

Where: R = H (26) [1 molecule per 14 bases]
R = OH (27) [1 molecule per 11 bases]

Hence from all this information, a proposed mechanism of action of MMC *in vivo* has recently been suggested which is illustrated on next page in *Scheme 7.*

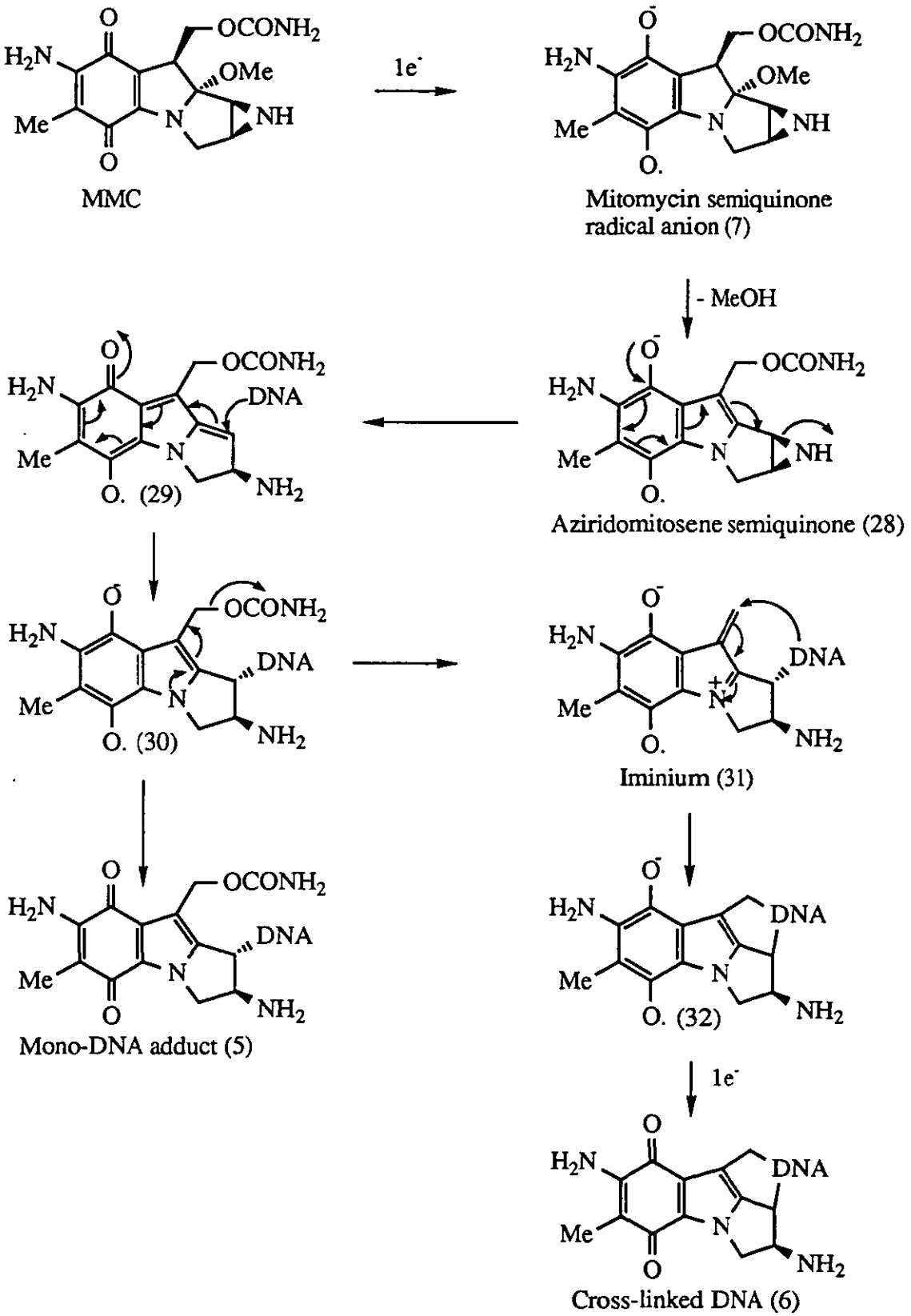
The initial step is a single electron reduction of MMC to the semiquinone radical anion (7). The radical anion (7) then readily loses methanol. The activated mitosene semiquinone formed (28) is now rendered electrophilic both at C-1 and C-10 by opening of the aziridine ring which is assisted by the radical anion as shown in *Scheme 7,* and by elimination of the carbamate group assisted by the "push" from the indole nitrogen, respectively. However, the first alkylation of DNA is thought to occur at C-1. Evidence for this comes from the isolation, after oxidation, of mono a C-1 DNA adduct, but not a mono C-10 DNA adduct. Hence, subsequent protonation and aziridine ring opening affords a reactive intermediate (29) capable of alkylation at C-1. The resulting compound (29) can be oxidised to the quinone, mono-DNA adduct (5), or since it is still a radical it can readily lose the carbamoyloxy substituent to afford an iminium compound (31) which can cross link to give a bis C-1/C-10 DNA adduct (6). In both the opening of the aziridine ring and elimination of the carbamate group, the products formed are attacked by nucleophiles, apparently by an S_N1 type mechanism. This appears to be a reasonable mode of action of MMC but now it is necessary to consider the different product profiles, by varying reducing agents, and also the role of the C-10 in the alkylation process.

1.4. Product Profiles by Varying (i) Reducing agent and (ii) Nucleophile and the role these have on Bis C-1+C-10 Adduct Formation

The product profile of the reductive activation of MMC depends on the; (i) reducing agent, (ii) nucleophile, (iii) concentration of oxygen or MMC itself. As already previously stated, the reductive techniques use chemical, catalytic hydrogenation, electrochemical or enzymatic methods.

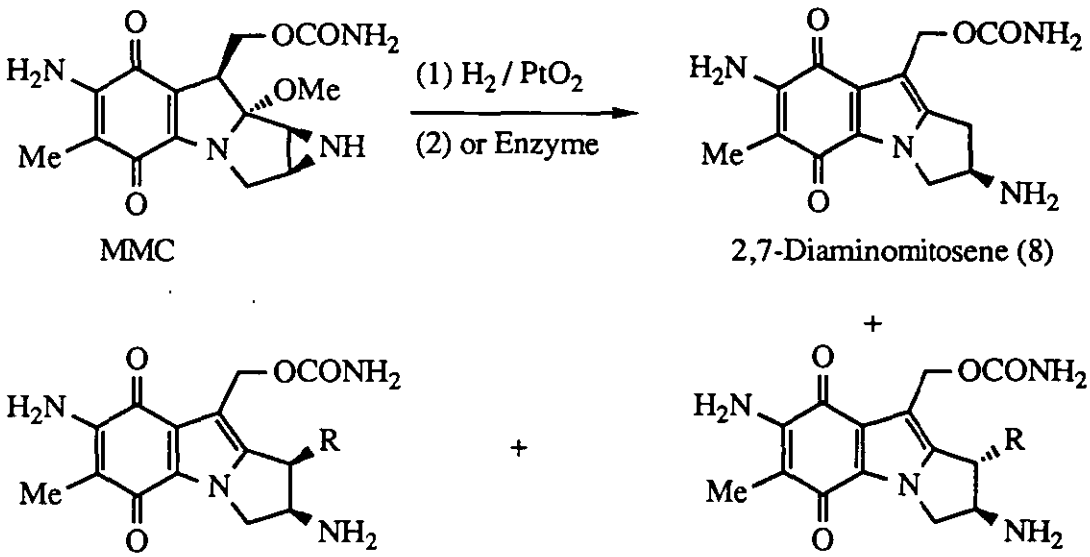
It is well documented in the literature, especially by the work of Tomasz and Kohn that under a variety of reductive conditions: enzymatic, chemical, electrochemical, and catalytic hydrogenation, activation of the C-1 in MMC proceeds rapidly at approximately neutral pH values (pH 7.0-8.5) to give the C-1 *cis* and *trans* nucleophilic substituted diastereomeric products, while under slightly acidic conditions (pH 5-6) C-1 electrophilic transformations predominate. However, when large nucleophiles such as 2'-deoxy-guanosine or DNA are used as nucleophiles *trans* products are predominately afforded. Also, it must be noted that conventional methods such as hydrogenation, electrochemical, chemical, and enzymatic normally give predominately C-1 mono-adducts and not the bis C-1+C-10 adducts. These points are evident by studies performed by Tomasz and

Scheme 7: The Proposed Mechanism for the Mode of Action of MMC



coworkers in which MMC was shown to form a covalent complex with calf thymus DNA. Under anaerobic conditions in the presence of either NADPH : cytochrome P450 reductase / NADPH, xanthine oxidase / NADH, or the chemical reducing system (hydrogen / platinum oxide) the DNA complex was digested with deoxyribonuclease I / snake venom diesterase / alkaline phosphatase, yielding a C-1 mono adduct which was isolated and characteristic by HPLC. The C-1 mono adduct (23) was formed in greater than 90% yield with only traces of the bis alkylation products (24) and (25).[29] Tomasz *et al.* also investigated the enzymatic reduction of MMC in rat liver microsomal systems, with sodium phosphate (the nucleophiles were supplied from the buffer) at neutral and acidic pH and the results are summarised in *Scheme 8 and Table 2*.[27]

Scheme 8



Where: R = OH; *cis* and *trans* 2,7-Diamino-1-hydroxymitosene: (9) and (10)
R = $OP(O)(OH)_2$; *cis* and *trans* 2,7-Diaminomitosene-1-phosphate: (11) and (12)

***Table 2:** Yields of (8) at various pHs*

pH	Yields (%)
5.1	52
7.0	15
9.0	0

C-1 mono diastereomeric adducts (9-12) were afforded with either phosphate or water as the nucleophile at neutral pH, but when the pH was lowered to 5.1 the predominant product was the C-1 electrophilic adduct (8).[27]

These types of C-10 modification processes are known to occur less readily than C-1 reaction due to the diminished reactivity of this site. The use of strong nucleophiles, such as potassium ethyl xanthate had provided access to C-10 nucleophilic substitution adducts. In the presence of base or in the absence of external nucleophiles the corresponding C-10 electrophilic products were obtained.[31, 32, 42, 43] These observations were noted by Hornemann and Kohn. When MMC was treated with sodium dithionite at approximately neutral pH: 0-5°C in the present of potassium ethyl xanthate the C-1+C-10 adducts were afforded in greater than 90% yield with the *cis*- and *trans*-1,2 disubstituted mitosenes in the expected 1:1 ratio.[32] Similar results were obtained when potassium ethyl monothiocarbonate was used as the nucleophile, except this time the reaction was conducted at room temperature.[42] This ratio of 1:1 for *cis* and *trans* gives more support for the S_N1 type mechanism. However, when potassium thiobenzoate was used as the nucleophile at approximately neutral pH this led to the formation of *trans* as the major product and also the principal site of alkylation of the MMC was determined to be C-1 (greater than 10:1).[43] In light of the similar reductive conditions utilised in all the sulphur based nucleophilic studies, the selectivity of the potassium thiobenzoate reactions has been attributed in part to the decreased nucleophilicity of the anion and enhanced the reactivity of C-1 versus C-10 in MMC.

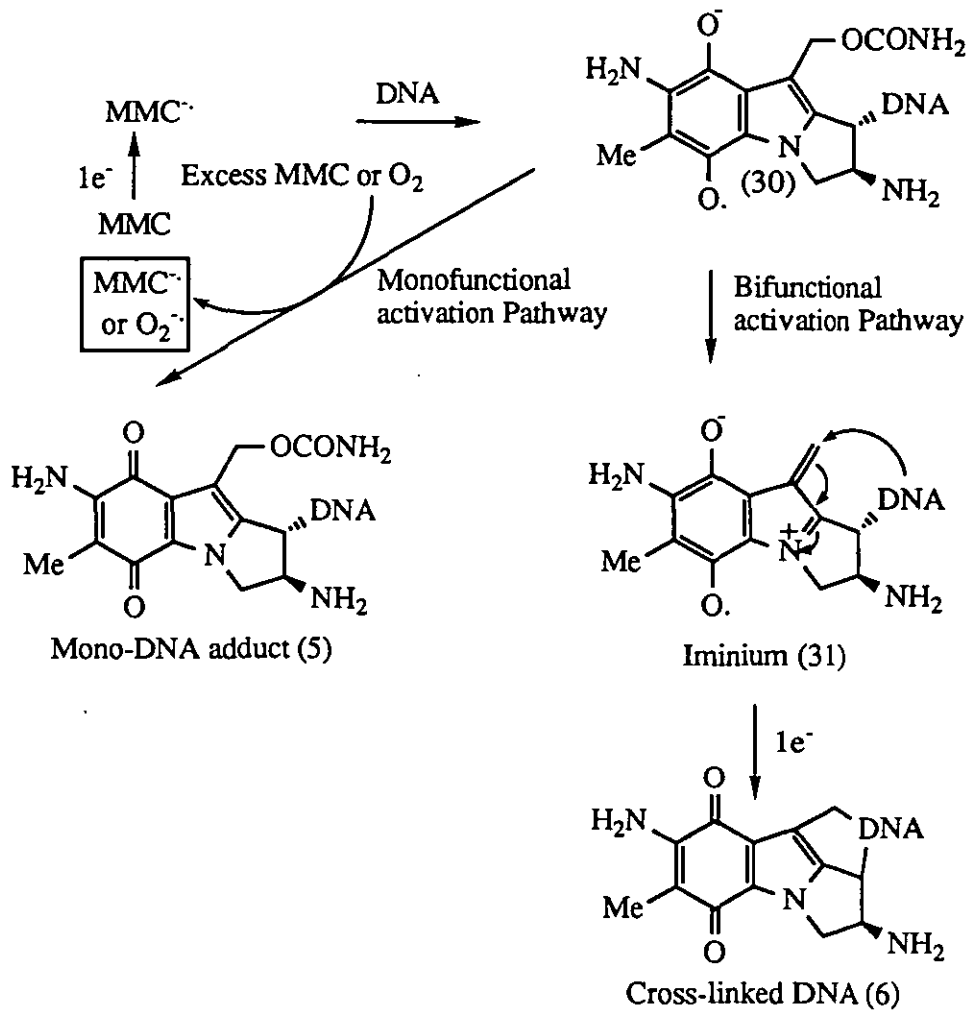
In this Section the mechanism of forming monofunctional and bifunctional MMC-DNA complexes will be discussed, with varying the reducing agents. As already discussed the work achieved by Tomasz *et al.* (1987),[30] showed three different adducts, one monofunctional (23) and two bifunctional adducts (24) + (25). The product distribution depended on the conditions of reductive activation *in vitro*. Monofunctional adducts were afforded when catalytic hydrogenation or enzymes were used, but bifunctional adducts were formed when sodium dithionite was the reductant. Tomasz investigated the reasons for these controversial results. In a set of experiments, which are summarised in *Table 3* various conditions such as; (i) reducing agents, (ii) the concentration of the catalyst and (iii) MMC, were varied.[44]

Table 3: Summary of Adduct Distribution under Various Reaction Conditions

MMC+DNA	Reaction Conditions	Adducts (23)	(24)+(25)
	H_2 / PtO_2, standard reaction	84%	16%
	H_2 / PtO_2, excess MMC	96%	4%
	H_2 / PtO_2, excess PtO_2	0%	100%
	Xanthite oxidase, standard reaction	95%	5% (24) 0% (25)
	Xanthite oxidase, excess NADH	88%	12%
	$Na_2S_2O_4$, standard reaction	0%	100%
	$Na_2S_2O_4$, air	53%	47%

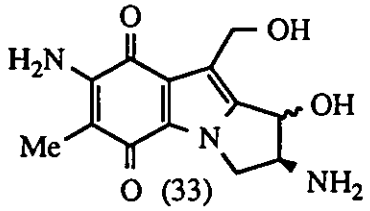
Hydrogenation over platinum oxide, observed in all previous studies acted as a monofunctional activating agent of MMC at C-1. However, by varying the conditions such that the catalyst (platinum oxide) was in excess bis C-1+C-10 adducts were afforded.[44] This was also seen to a lesser extend with xanthine oxidase / NADH which switched from a purely monofunctional adduct (23) to a partly bifunctional adducts when the enzyme + NADH / MMC ratio was increased. In contrast, with a large a excess of MMC bifunctional adducts (24) and (25) were dramatically suppressed. These different product profiles have provided new information concerning the factors which control the C-1 and C-10 substitution process of the anticancer agent. The authors suggested that the mechanism featuring reduction kinetics rather than redox potential as the critical factor. Peterson and Fischer's discovery of the autocatalytic activation process of MMC was important in explaining this proposal.[45] In such a process a catalytic amount of electron equivalents induces stoichiometric activation of MMC in a chain reaction to yield monofunctional mitosene as the final product (5). The authors postulated that after an initial reduction and an alkylation step the mono adduct species (30) as shown in *Scheme 9* can follow two reactive pathways; (i) electron transfer to unreacted MMC (autocatalytic conditions), hence losing its activated state and giving mono adduct (5), or (ii) retro-Michael elimination of the C-10 carbamate group to give (31), which can trap a nucleophile such as DNA, thereby giving a bisalkylated end product (6). The experimental results constitute proof for the concept that underlies the mechanism in *Scheme 9*.[44] This predicts that if the reduction rate is slow, the monofunctional, autocatalytic activation pathway predominates because (30) is inactivated by excess MMC faster than it is formed. Alternatively, if the reduction rate is fast, as in the case with excess platinum oxide, (30) will accumulate and have a long enough lifetime to undergo the second activation step.

Scheme 9: The Mechanism of Monofunctional and Bifunctional Alkylation of DNA with MMC

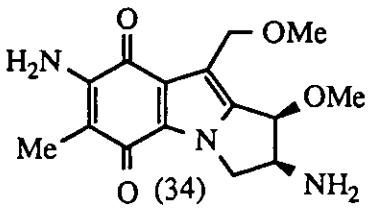


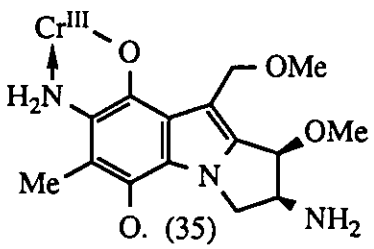
Kohn has recently introduced into the list of reducing agents, a novel transition metal, 1-electron reductant, chromium (II) perchlorate. This has dramatically altered the reactivity pattern of the two DNA binding sites within MMC, depending on which chromium (II) perchlorate-mediated reductive technique was used.[8, 46, 47] The product profiles using these two techniques were dramatically different indicating that different pathways for the MMC activation process existed in the two procedures.[8, 46] In the first method, activation of MMC was achieved by the direct addition of standardised aqueous solution of chromium (II) perchlorate (1-2 equivalents) to a degassed, buffered, aqueous solution of the MMC at various pH values. It was observed that consumption of MMC was rapid and generated both *cis*- and *trans*-10-decarbamoyl-1-hydroxy-2,7-diaminomitosene (33) as the major products, and the reaction efficiency increased at lower pH values. It is interesting to note that no significant amount of C-1 electrophile

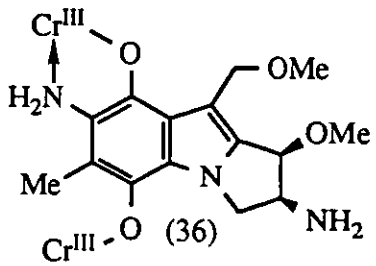
products were observed under acidic conditions. These observations were opposite to that normally reported for the activation of MMC with other reductive methods.



The second chromium (II) perchlorate-mediated MMC activation procedure (indirect reductive procedure) was achieved by prior addition of chromium (II) perchlorate to excess *cis*-10-decarbamoyl-1,10-dimethoxy-2,7-diaminomitosene (34) to generate, the monochromate (35) and dichromate (36) species *in situ*, followed by the addition of MMC (1 equivalent per chromium perchlorate). This time, the product distribution obtained in these experiments was similar to those previously reported, particularly the percentage of C-1 electrophilic adduct (8) generated, which was dependent upon pH. This gave predominately C-1 monosubstituted products (9) and (10) as shown in *Scheme 10*.

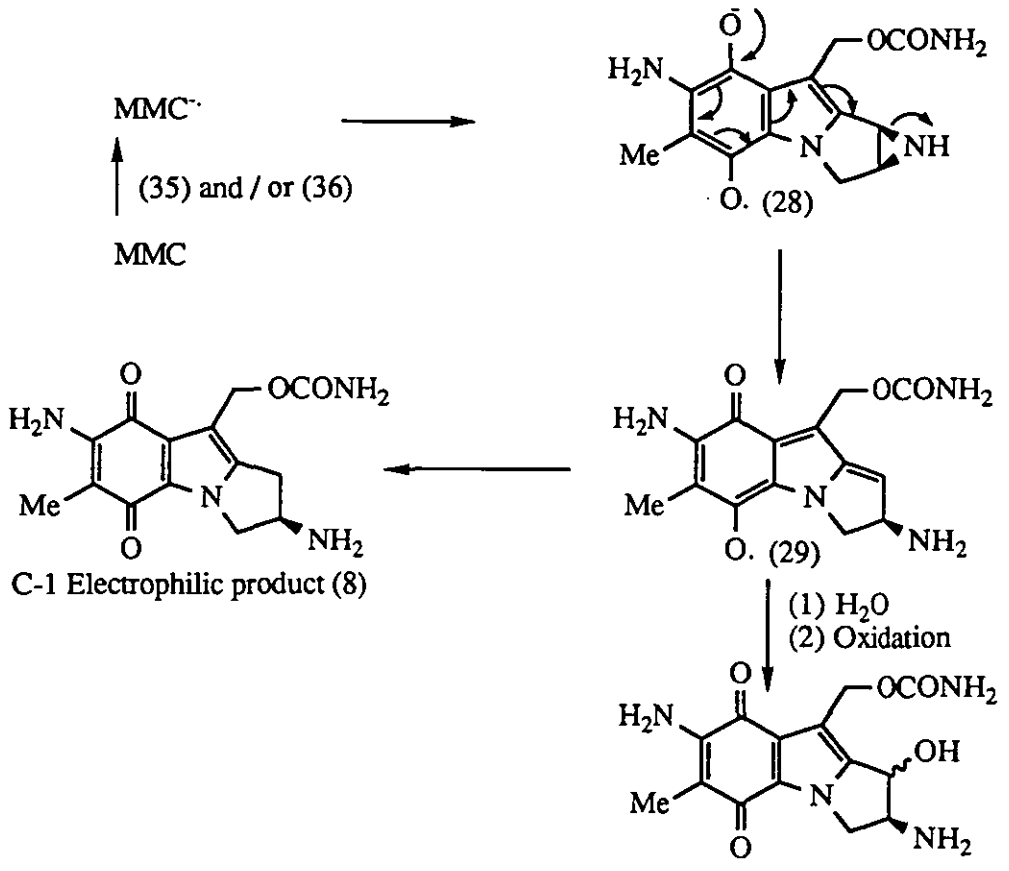






What factors are responsible for this divergent chemistry? The authors suggested two different mechanisms for the activation of MMC. Firstly, they postulated that the indirect reductive procedure followed a mechanism similar to that previously proposed in *Scheme 7* as shown in *Scheme 10*.

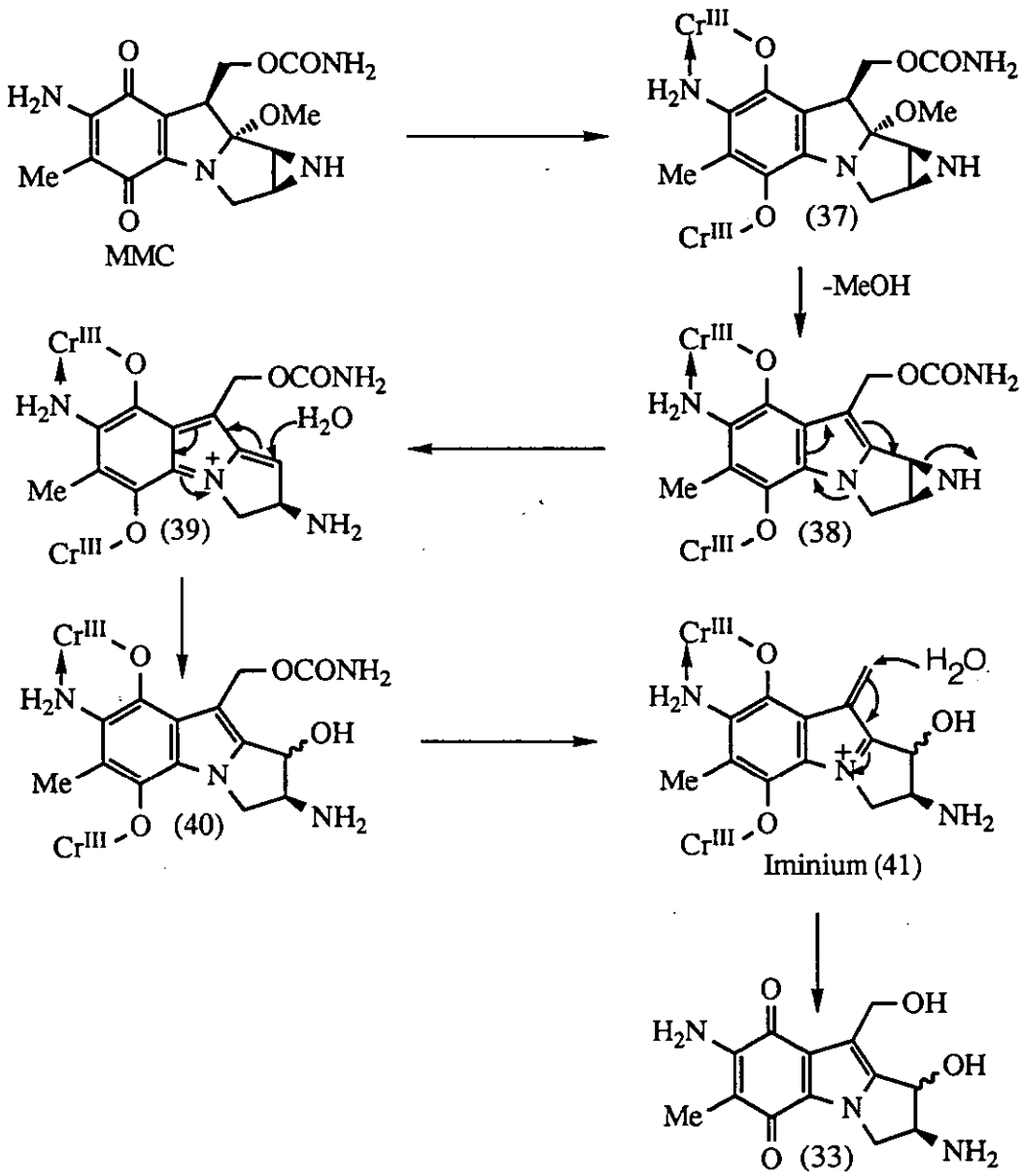
Scheme 10: Proposed Pathway for Mitomycin C Activation at C-1 Using $Cr(ClO_4)_2$



C-1 Nucleophilic *cis* and *trans* products: (9) and (10)

Reductive activation of MMC by an indirect method occurs *via* an outer-sphere electron transfer process from (35) and / or (36), to give the uncomplexed MMC semiquinone radical anion (7).[46] However, in a contradicting paper, the authors suggested that the hydroquinone (2) was formed.[8] The authors proposed for the direct method that the rapid 2-electron reduction of the quinone ring of the MMC led to a bis-Cr^{III} bound complex (37) as shown in *Scheme 11*. Loss of methanol afforded (38) which allowed the C-1 and C-10 nucleophilic substitution processes to proceed by an indole-assisted pathway as shown in *Scheme 11*. They suggested that the high percentage of nucleophilic C-1+C-10 product in these transformations was due to the complexation of the C-5 and C-8 phenolic-type oxygen with Cr^{III}.

Scheme 11: The Predominant Pathway for the Reductive Activation of MMC by the Direct Addition of $Cr(ClO_4)_2$ in Water



Similar results were also obtained with methanol.[8] The authors have also extended this direct method for the generation of C-1+C-10 substituted mitosenes, which gives a higher yield of bis C-1+C-10 nucleophilic substituted mitosene adducts than most other reductive activation techniques. The proposed mechanism by Kohn is in contrast to that of Tomasz for bisalkylation at C-1+C-10. These species are very short lived and it is very difficult to isolate any intermediates. Both mechanisms appear to make sense in their own right but it should be noted that Tomasz suggested a semiquinone radical anion intermediate (30) whereas Kohn postulated a two electron reduced species (37)

complexed to chromium to afford the bifunctional C-1+C-10 adducts. It is necessary to investigate the controlling factors of C-1+C-10 bifunctional versus C-1 monofunctional processes so that a clearer picture of the mechanism of action emerges. The direct chromium (II) perchlorate method has proven useful for bonding of MMC to reagents of varying nucleophilic strength, such as, water,[46] methanol,[8] 2'-deoxyguanosine, and aniline.[47] This is a novel method for the formation of the C-1+C-10 bis MMC adduct.

In Summary: In this Section we have discussed the evidence for the semiquinone radical anion (7) as opposed to the hydroquinone (2), first postulated by Iyer in 1964.[19] This is still a very controversial issue but in light of the experimental evidence, the semiquinone radical anion seems most likely. It might also be probable that both the semiquinone radical anion (7) and hydroquinone (2) play a role in the mode of action of MMC but at different times. Recently, a considerable amount of work has been concerned with determining what factors control C-1 monofunctional adducts in contrast to C-1+C-10 bifunctional adducts in MMC, by varying (i) reducing agents (ii) concentration of MMC, as shown in the previous examples. This is a complex field and considerable investigation must still be conducted to further our understanding of the mechanism of action of MMC, both *in vitro* and *in vivo*.

1.5. Binding Sites with DNA

There are several different conformational forms of DNA. These are known as A, B, D or Z-DNA (the later is a left handed helix), of which B-DNA is the most common form. MMC or its metabolites must make some kind of initial non covalent interaction with the DNA. Early studies on the alkylation of DNA by MMC, gave chiefly on degradation, fragments which contained predominately *trans* 2,7-diaminomitosenes structures with purine bases, *i.e.* guanine (G) or adenine (A) attached at C-1.[48] The preference order of alkylation of bases was established as O(6) guanine, N(6) of adenine, and 2-NH_2 group of guanine, the latter being least favourable.[49] Recently, Tomasz isolated the cross-linked C-1+C-10 adduct (25) and mono-linked C-1 adduct (23), which were attached at C-1 and C-10 to 2-NH_2 of guanine.[30] One year later M^c Guinness and Nakanishi synthesised a guanine derivative substituted at the O(6) position by MMC.[50] The authors pointed out previous authors assignment of O(6) guanine-MMC was not compatible with the properties of the authentic adducts which M^c Guinness and Nakanishi had synthesised. Therefore, the authors concluded that the 2-NH_2-adducts of guanine were the major products obtained upon reductive activation of MMC. These observations

confirmed that alkylation occurred at C-1 and C-10 *via* the 2-amino group of the guanine residues. For the moment, leaving these observations and concentrating on the Watson-Crick DNA base theory, computer modelling studies predict strong non-covalent binding at all three of these sites. Further studies revealed that covalent binding between MMC and the N(6) position of adenine (in the major groove) or the 2-amino group of the guanine (minor groove) can proceed without disrupting the Watson-Crick hydrogen bonding framework between base pairs. However, binding of the O(6) of the guanine (major groove) results in partial disruption of the system and it must be energetically less favourable. Tomasz *et al.* proposed a snug, relatively non-distortive fit for both the monofuntional adduct (23) and the cross-link adduct (25) in the minor grove of duplex B DNA, based on physicochemical studies and computer-assisted energy minimized modeling.[51]

Recently, Tomasz, Crothers and other workers have provided evidence that not only is the reduced MMC highly selective to the 2-amino group of guanine but often a higher order of selectivity is imposed by the base sequence around the guanine. Crothers and coworkers reported, using an extended series of duplex dodecanucleotides and isolating by gel electrophoresis, that MMC cross-links *in vitro* occurred selectively between guanine in CG.CG in strong preference over GC.GC and no significant cross-linking was found when G was preceded by any nucleotide other than C (deoxycytidine-5'-monophosphate, Appendix 1).[52] Furthermore, cross-linking per CG.CG sites arose when there were several such sites in series. The authors tried to rationalised these observations by using the Amber program which compared the energy of the oligomers crossed-linked at CG.CG and GC.GC sites in the B DNA geometry. However, according to the calculations, CG.CG and GC.GC are energetically similar and hence the difference in the reactivity can not be rationalised by energy miminisation of the final products.

Tomasz *et al.* have extended this work.[53] In this study an extensive series of oligodeoxyribonucleotides was reacted with reductively activated MMC, and the resulting cross-linked drug-oligonucleotides complexes were again isolated by reverse-phase HPLC. The authors, performed three different experiments. Firstly, in comparison with Crother's work, AT-rich duplex oligonucleotides, containing only a single central CG.CG (Appendix 1), gave high yields of cross-links between the guanine while those having GC.GC, instead, gave none, as shown below in *Table 4*. In the second experiment, alternative replacement of the deoxyguanosine by deoxyinosine in the CGC.GCG sequence, showed that CGC.GCG and CGC.ICG both yield approximately 50% cross-link while CGC.GCI was completely resistant. When the oligonucleotide had

two adjacent CG.CG sequences the percentage yield of cross-linking was enhanced as illustrated below in *Table 4*. In the third experiment, Tomasz confirmed that the two guanines were cross-linked to interstrands of DNA.

Table 4: *Comparison of Yields of Cross-Links at CG.CG and GC.GC Sites Oligonucleotides Duplex*

Sequence	% Yield of Cross-Link	Comment
5'-TATATCGATATA 3'-ATATAGCTATAT	19	
5'-TATATGCATATA 3'-ATATACGTATAT	0	
5'-ATATACGTATAT 3'-TATATGCATATA	47	
5'-ATATACGCTATA 3'-TATATGCGATAT	47	5' CGC formed 3' GCG
5'-ATATACGCTATA 3'-TATATICGATAT	0	No cross-links formed
5'-ATATACGCGTATAT 3'-TATATGCGCATATA	80	Only one cross-linked formed

Note: These are the simple representations of deoxyribonucleotides which are always written with the 5' end at the left and the 3' end at the right *i.e.* the 5'-3' direction (for a single strand of DNA-Appendix 1)

In Summary: From recent observations C-1 and C-10 of MMC are linked to the 2-amino group of guanine and furthermore CG.CG sequence is absolutely required for MMC cross-link formation. Also shown was the fact that cross-linking occurred between interstands of DNA and not intrastrands. However, more investigation is necessary to rationalise these observations and to understand the mode of action of how MMC binds to DNA at specific sites, and the factors which control these.

1.6. Bioreductive Drugs in Cancer Therapy

1.6.1. Tumour Hypoxia

Solid tumours consist of aerobic and hypoxic (anaerobic) fractions, which account for somewhere between 10-25% of the total tumour volume (the exact percentage is not known). The hypoxic fractions of solid tumours are widely believed to contribute to the limitation of radiation and chemotherapy in the treatment of neoplastic diseases. A basic principle of the selective cancer therapy is that the treatment should kill the tumour cells while at the same time spare the normal tissues. For this reason bioreductive agents appear to be attractive from the view of antitumour selectivity *via* (i) tumour hypoxia and (ii) the expression of the reductive enzymes, such as DT-diaphorase or NADPH : cytochrome P450 reductase.

1.6.2. Types of Bioreductive Drugs

Bioreductive antitumour agents are prodrugs. That is they are inactive in their own right, but are able to undergo metabolism to species which damage biomolecules upon metabolic reduction. Many drugs with widely different chemical structures have this ability to undergo bioreductive activation in such a way as to selectively kill hypoxic cells. Examples of these are; (i) quinone alkylation agents, (ii) various nitroheterocycles and (iii) the benzotriazine di-*N*-oxides.[54] The chemical structures of these compounds are shown in *Figure 2, 3, 4* respectively.

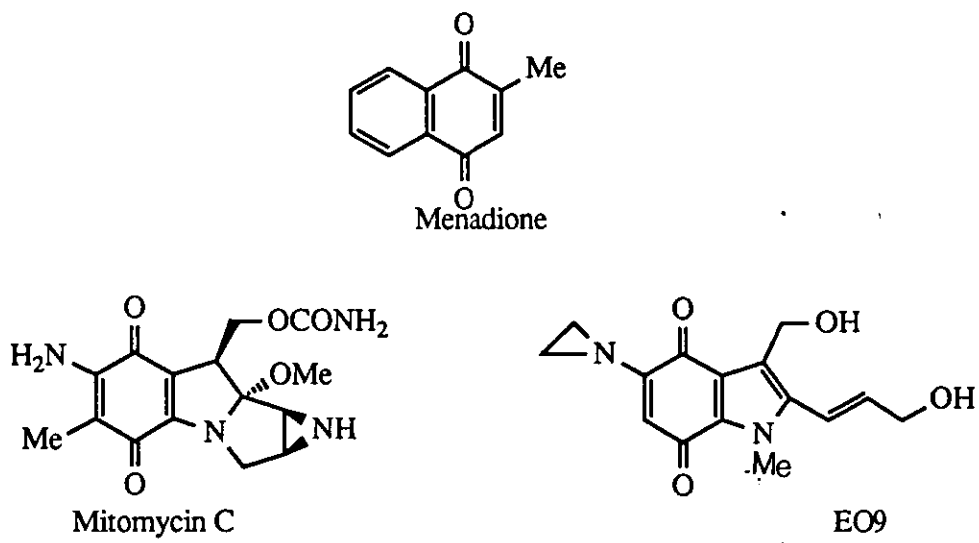
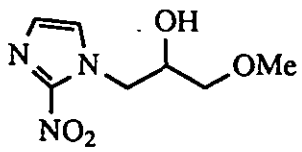
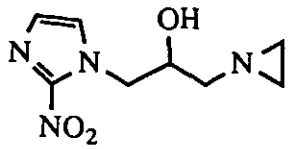


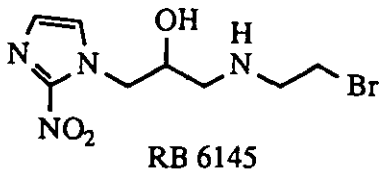
Figure 2: *Structure of menadione and two bioreductive quinone alkylating agents that undergo bioreductive activation to act as hypoxic cell cytotoxins*



Misonidazole

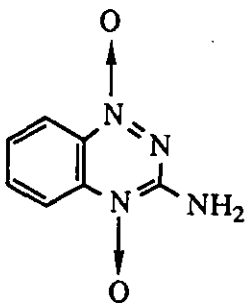


RSU 1069



RB 6145

Figure 3: *Structures of three nitroheterocyclic compounds that are so-called "dual-function" agents which can act as radiation sensitisers and also as bioreductive hypoxic cell cytotoxins*



SR 4233

Figure 4: *The structure of benzotriazine di-N-oxide SR 4233*

Depending upon the chemical structures, bioreductive drugs can show four distinct mechanisms of action, although these are not necessarily operative in all such drugs. Two mechanisms (i) radiosensitisation and (ii) hypoxic cytotoxicity will be discussed here.

Radiosensitisation [55]

The major mechanism of hypoxic cell radiosensitisation is oxygen-mimetic in character. The agents must be present at the time of irradiation and free-radical mechanisms are involved. Further, the overall effect is to increase the efficiency of radiation-induced DNA damage. Sensitisation is usually not observed in oxygenated cells and this is the basis for tumour selectivity.

Hypoxic Cytotoxicity [55]

The phenomenon was first observed in multi-cellular spheroids treated with metronidazole,[56] and was soon followed by examples of differential hypoxic cytotoxicity

induced by misonidazole and by other nitroheterocycles.

This mechanism is very different from radiosensitisation. The process is much slower, high temperature-dependent and shows a much greater cell-line variability than in the case for hypoxic cell radiosensitisation. The key step, as already stated, in this process is enzyme-mediated reductive activation. Reductases involved include DT-diaphorase, cytochrome P450, NADPH : cytochrome P450 reductase, and xanthine oxidase. The ease of bioreduction of a given drug will depend on (i) the ability of the drug to act as a substrate for the enzyme(s) and (ii) expression levels of these enzymes in a particular cell type. Also, depending upon whether or not the drug is reduced in a one or two electron step, the initial step in the reduction may be reversed by oxygen. All these factors contribute to the variability of cytotoxic effects observed in different cell types, and also the variable magnitude of the hypoxic cytotoxic differential. Now it is necessary to discuss the reductases and this bioreductive activation reaction.

1.6.3. Detailed Look at the Bioreductive Activation Reaction

Figure 5: *Bioreductive Activation with Possible Intermediates*

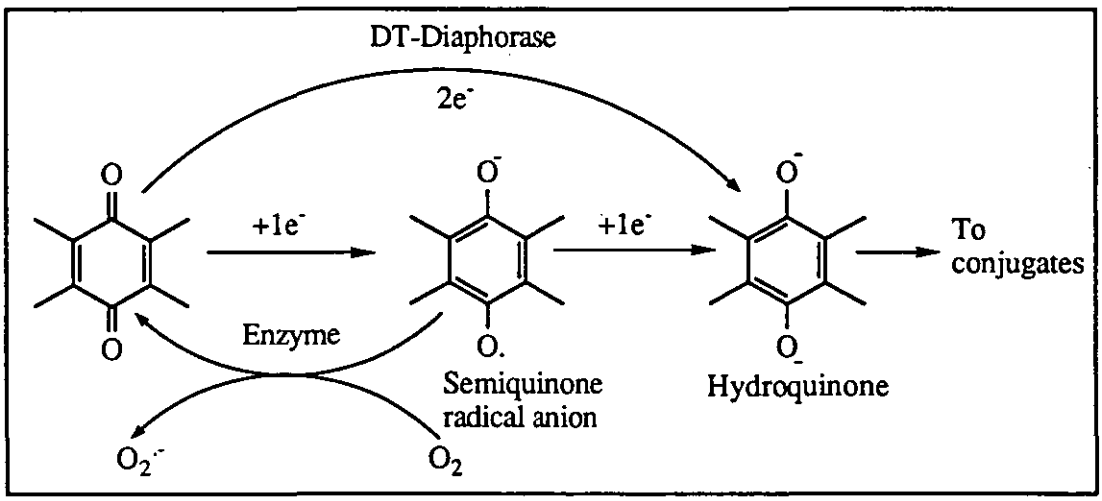


Figure 5 shows the classic model for the reductive metabolism of a simple quinone such as menadione under aerobic conditions.[57] The aerobic toxicity of such compounds arises *via* one electron reduction to the semiquinone free radical by an enzyme such as NADPH : cytochrome P450 reductase. This leads to toxic superoxide radicals in the presence of oxygen to generate a futile cycle, and the cell is placed under what is known as "oxidative stress", leading eventually to DNA fragmentation. The effect of this superoxide radical is reduced by the action of protective enzymes such as superoxide dismutase and catalase

which detoxify superoxide. Another pathway that protects oxic cells (oxygenated) from the toxic action of quinones is direct reduction by the unusual DT-diaphorase enzyme. This catalyses an obligate 2-electron reduction rather than the 1-electron reduction, hence bypassing the semiquinone radical anion. Therefore the opportunity for futile cycling is reduced, especially when the resulting hydroquinone is conjugated and then eliminated. In this situation DT-diaphorase will act as a bioprotective enzyme against oxidative stress. The picture is yet more complex for quinones possessing appropriate alkylating or reactive leaving groups. Under aerobic conditions cytotoxicity may arise from both free radical formation and DNA alkylation,[38] whereas under hypoxic conditions, alkylation reactions will predominate.

1.6.4. DT-Diaphorase

DT-diaphorase is a predominately cytostolic enzyme.[58] Activity has also been detected in a number of subcellular organelles including endoplasmic reticulum, mitochondria and Golgi apparatus. The enzyme is widely distributed among organs, with particularly high levels being expressed in the liver, kidney, and gastrointestinal tract.[58] The enzyme exists as a dimer of molecular weight approximately 55,000 and contains two subunits of unit size and two molecules of FAD. This enzyme lacks specificity for either of the two nicotinamide dinucleotides, and the enzyme is inhibited by dicoumarol.[58] This enzyme has the potential for either detoxification against "oxidative stress" as seen above or activation to DNA damaging species-bioactivation as discussed later. Hence in view of its potential for either activation or detoxification, DT-diaphorase can be seen as a two-edged sword with respect to bioreductive drug development.[38, 57, 58]

1.6.5. Bioreductive Drugs

MMC as previously discussed.

EO9

EO9 is the leading compound in a novel class of indoloquinones.[55] The drug is structurally related to MMC and is about to enter clinical trials as an anticancer drug with EORTC (European Organisation for Research and Treatment of Cancer). E09 has various characteristics, (i) a broad spectrum of activity against human tumour cell lines, which is a little different than MMC and (ii) a lack of myelosuppression, which is a big side effect of MMC. EO9 is considerably more active than MMC under both aerobic and anaerobic conditions. Also the hypoxic / oxic differential of approximately 30 for EO9 is greater than that for MMC (2) against KHT murine tumour cell line *in vitro*.[55] A similar

bioreductive activation, as with MMC, is thought to occur with EO9. Here reduction will activate the hydroxyl leaving groups and the aziridine ring, generating a total of three reactive centres per molecule, as a result of bioreductive metabolism. Recently, work has been done by Workman *et al.* to establish what enzymes are important in the bioreduction activation of this drug. A summary of some of the results are presented. Recent results have shown that EO9 is metabolised by DT-diaphorase in rat Walker tumour and human HT 29 tumour cells (which are rich in the enzyme).[59] The rat enzyme reduced EO9 at a much faster rate than the human enzyme, and the respective apparent Km values of around 3 and 15 μM [Appendix 2] suggested that EO9 may have a reduced affinity for the human enzyme-DT-diaphorase. Using highly purified quinone reductase or DT-diaphorase preparation from the rat Walker 256 tumour cell line, EO9 was found to undergo reductive bioactivation under aerobic conditions *in vitro* to give a reactive species which produced single-strand breaks in pBR 322 plasmid DNA (*i.e.* Walker tumour cells was able to metabolise EO9 to a species that caused supercoiled plasmid pBR 322 DNA to be converted to relaxed, circular conformation).[59] Also recently it has been shown that EO9 metabolised by DT-diaphorase can cause DNA cross-links in Walker tumour cells. It should be noted, that in contrast to MMC, the metabolism of EO9 is not pH dependent in the range of 5.8 to 7.8.[59]

RSU 1069

The dual function compounds as shown in *Figure 3* are the 2-nitroimidazoles containing potentially alkylating group in the N_1 side-chain. The leading compound is RSU 1069, an aziridinyl derivative of misonidazole.[60] As with the quinones, the bioreductive metabolism of these nitro compounds is quite complex. Reports in the literature from biologists suggest that the important metabolites appear to be the nitro radical anion (1-electron), the nitroso (R-N=O, 2-electron), and the hydroxylamine (R-NHOH, 4-electron). They suggest that these are likely to be toxic, whereas the amine (6-electron) is regarded as non-toxic.[54] However, chemists such as Bowman suggest that this is incorrect, and a better rationalisation is as follows.[61] The nitro radical anion has been observed *in vitro* and the structures have been studied. Studies indicated that the radical anions do not interact with DNA, and that the hydroxylamine, and amino-imidazoles are not active, and that the only active reductive metabolite is the nitroso. The aziridinyl nitromidazole RSU 1069 is considerably more potent *in vivo* than the simple nitroimidazole molecule. This molecule was designed so that after bioreductive activation of the nitro group the aziridine moiety can alkylate with DNA to generate a toxic cross-link. This confers extra potency over monofunctional nitro compounds and also enhances hypoxic cell specificity. Reduction of this compound is primarily brought

about by NADPH dependent P 450 reductase. RSU 1069, has radiosensitising properties but can be up to 100 times more toxic to hypoxic compared to aerobic cells.[60] This drug, can selectively target hypoxic tumour cell populations that are resistant to both radiation and to drug based therapies.

SR 4233

SR 4233 as shown in *Figure 4* is the leading compound in a series of highly selective hypoxic cytotoxins, the benzotriazine di-*N*-oxides, which exhibit improved efficacy and increased cytoxicity compared with its predecessors, the mitomycins and nitroimidazoles.[55, 58] In some cell lines the differential toxicity can exceed one hundred. SR 4233 shows high selectivity towards hypoxic mammalian cells *in vitro* and appears to be an effective antitumour agent *in vivo* when combined with radiation, or compounds which enhance tumour hypoxia. The principal reductaseswhich catalyse this reductive bioactivation are cytochrome P450 and NADPH : cytochrome P450 reductase.[58] The hypoxic cytotoxic action of SR 4233 is due to the one-electron reduction to give a free radical with oxidising properties.[58] SR 4233 causes double strand breaks to a much greater extent in hypoxic cells. However, in contrast to the other bioreductive quinone alkylating agents and nitro compounds, SR 4233 does not bind to cellular macromolecules such as protein and DNA.[55] Recently Workman and coworkers have shown that SR 4233 may be metabolised by DT-diaphorase from the Walker 256 rat carcinoma to both the 2 and 4 electron reduced products.[58] However, like MMC, SR 4233 does not appear to be a particularly good substrate for DT-diaphorase. Therefore, maybe DT-diaphorase may provide an important cellular defense against this novel hypoxic cytotoxic drug.

1.6.6. Enzyme Profiling: To Direct The Use of Bioreductive Drugs

The potential usefulness of the hypoxia directed bioreductive agents MMC, EO9, RSU 1069, and SR 4233, as illustrated above, will be enhanced by identifying appropriate human tumours for treatment, and also the expression levels of the enzymes in these tumours. Levels of DT-diaphorase are often far greater in some tumours than in normal tissues.[58] Once the bioreductive drugs have been identified to be good substrates for a particular enzyme, *i.e.* DT-diaphorase, and this enzyme in expressed in the tumour of interest, then this has potential for good treatment. Workman is presently working on these ideas and it is called the enzyme directed approach which is discussed below.[57]

The antitumour quinone MMC compared to EO9 was a poorer substrate with rat tumour DT-diaphorase, exhibiting a remarkably high Km and a Vmax only 5% of that for EO9.[38]

In fact, now it is apparent as mentioned earlier that MMC is only a poor substrate for DT-diaphorase at physiological pH, although metabolism of MMC by DT-diaphorase can be enhanced at acid pH. SR 4233 was shown to be preferentially reduced to the 4-electron product [58] by rat tumour DT-diaphorase, and it probably represents a detoxification pathway. In conclusion, the present results show that DT-diaphorase can function as either bioactivative (convert into cytotoxic metabolites) or bioprotective reductases, depending on the bioreductive drug involved. Hence tumours rich in DT-diaphorase should represent appropriate targets for EO9, and perhaps MMC, but be resistant to SR 4233. In such cases with patients with tumours expressing high levels of DT-diaphorase, SR 4233 might not be the most suitable bioreductive agent.

In Summary: Bioreductive activation provides a novel, exploitable pathway for the design and development of new anticancer drugs. Furthermore, if the enzymes that catalyse these reactions are identified and can be manipulated in suitable tumour types for treatment, then bioreductive drugs will have a large part to play in cancer therapy in the future.

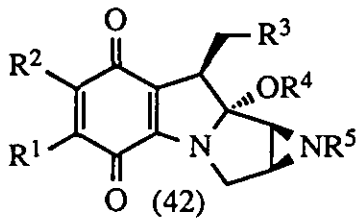
CHAPTER 2

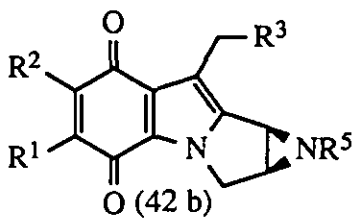
SYNTHESIS OF CYCLOPROPAMITOSENES AND MITOSENES

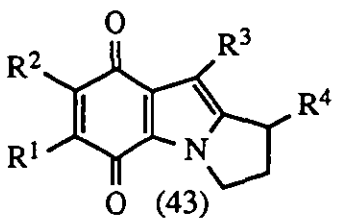
2.1. Introduction

As illustrated in Chapter 1, MMC is clinically a very useful chemotherapy drug for the treatment of a wide range of neoplastic conditions. Unfortunately, this drug has one major side effect, myelosuppression. Many analogues of MMC have been synthesised, but despite extensive efforts in this field only one analogue, EO9 has proved superior to MMC. Hence there is potential for a novel indoloquinone with enhanced antitumour activity to be discovered.

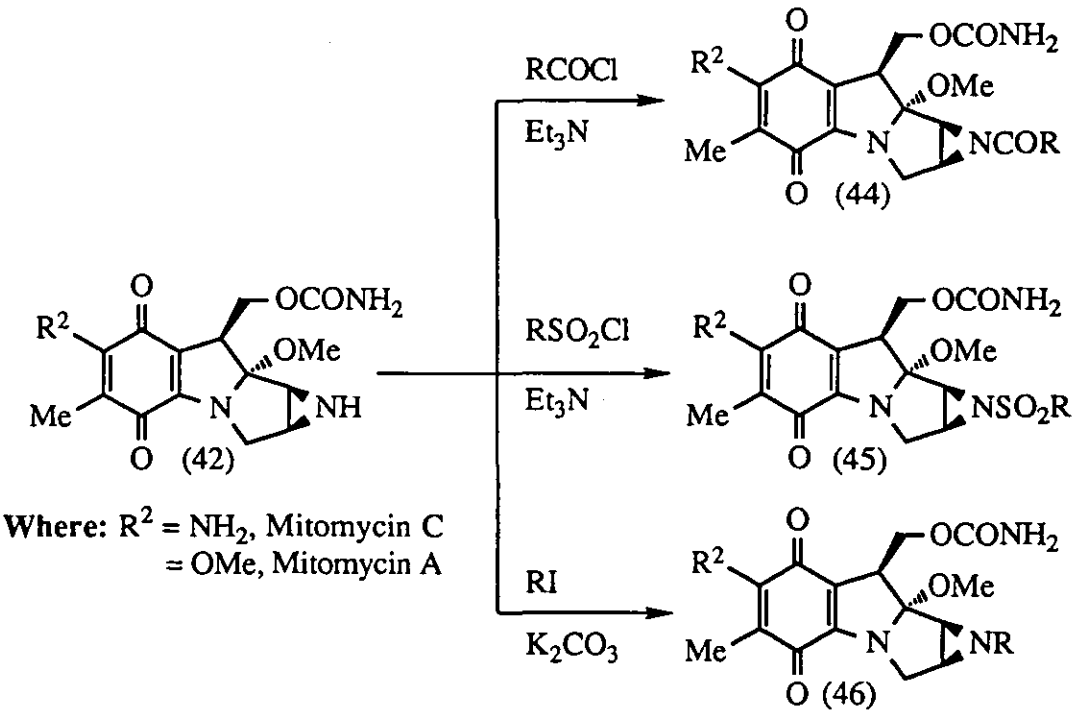
Analogues of MMC have been classified in three groups; (i) mitosane (42), (ii) aziridinomitosene (42 b), and (ii) mitosene (43) compounds, as illustrated below.





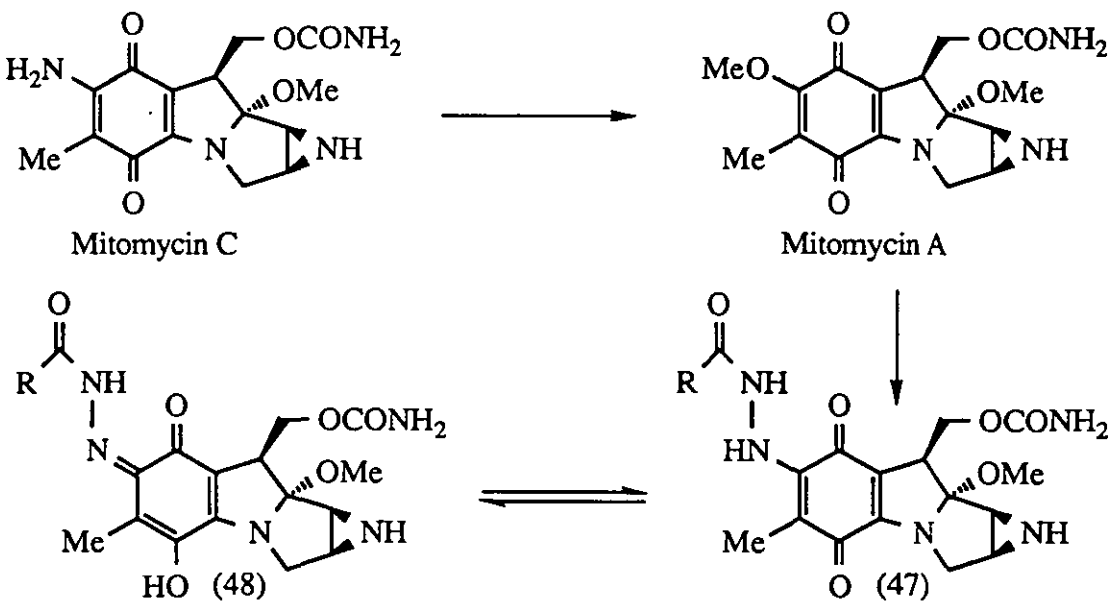


Firstly, reactions of mitosanes (42) will briefly be discussed. The aziridino-nitrogen of MMC and MMA can be acylated to afford (44) under mild conditions with acyl chlorides in triethylamine.[62] Among the acyl groups that have been substituted on the aziridino-nitrogen are (i) acetyl, (ii) other alkanoyls, and (iii) substituted benzoyls. Sulfonyl derivatives (45) of MMC and MMA are readily prepared from the corresponding sulfonyl chloride.[62] Alkylation of the 1a position of mitomycins was achieved from alkyl iodide in the presence of potassium carbonate to generate (46),[62] as illustrated below.



Where: $R^2 = NH_2$, Mitomycin C
= OMe, Mitomycin A

MMC derivatives with various substituent groups at the C-7 position have also been synthesised because position 7 controls the reduction of the quinone ring. This, offers a chance to gain some selectivity between normal and certain cancer cells. Kohn *et al.* synthesised an unusual group of MMC analogues which were substituted with a hydrazide at position 7.[63] MMC was first converted to MMA and then treated with 2 equivalents of acid hydrazide or alkyl carbazate in methanol to afford (47), which tautomerises. Evidence that these analogues existed as the *o*-azaquinone tautomer (48) in the solution state, as shown below [63] is based on several key spectroscopic observations; (i) compounds all displayed a pronounced absorption in the visible spectrum between 376 and 395 nm, (ii) carbon-6 methyl protons consistently appeared downfield (δ 2.1) from the typically observed value for MMC adducts (δ 1.8), (iii) ^1H NMR spectrum of one of the compounds in CD_3CN revealed a peak at δ 17.03 integrating for one proton (normally associated with acidic hydrogens-phenolic, carboxylic acid protons).

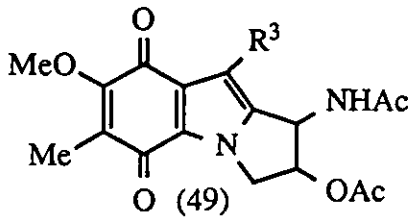


O-Azaquinone Tautomer
Where: R = Alkyl, aryl, and alkoxide groups

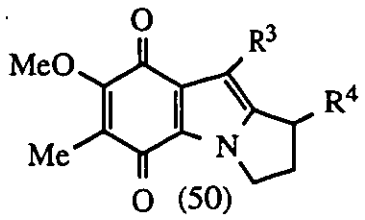
MMC analogues with different alkoxides and amines at the C-7 position have been synthesised, and these will be discussed in Chapter 3.

Mitosene analogues of MMC have also been synthesised. Remers *et al.* have synthesised a range of mitosenes of the general form of 1,2-disubstituted (49) [64] and 1-substituted (50) [65] as shown below. The 7-methoxy group was also exchanged for

other groups such as amines.[65] The authors suggest that in general mitosene (50) analogues with moderately good leaving groups (mostly esters) at the C-1 position were active, whereas analogues without such substituents were inactive or barely active. These results lend support to the idea that mitosenes with leaving groups at positions 1 and 10 are capable of bifunctional alkylation of DNA in a manner similar to that of MMC.

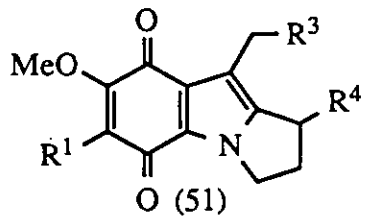


(49)

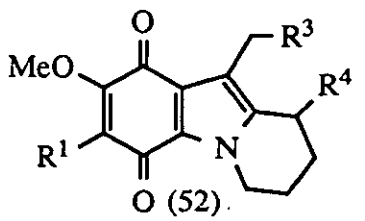


(50)

This idea was also confirmed by Orlemans.[66] This group was working on mitosenes of the general structures (51) and (52), as shown below.



(51)



(52)

Remers *et al.* state that for a bioreductive activation an optimally functionalised mitosene derivative should have two leaving groups, both at position 1 and 10. It was also observed that pyrido[1,2-a]indoles (52) exhibit lower activity than the corresponding analogous pyrrolo[1,2-a]indoles (51). It is difficult to explain the difference in biological activity between the five and six membered mitosenes. The lower activities of the pyrido[1,2-a]indoles (52) derivatives may be ascribed to unfavourable steric or other factors imposed on these compounds by the additional methylene moiety.[66] All the MMC derivatives were biologically tested, and in the indoloquinone family, only one drug superior to MMC is being developed as a neoplastic drug, EO9.

However, Moody and Jones proposed a novel idea.[67, 68] Recently, the authors diverted totally from other previous ideas and proposed the rationale to synthesise novel compounds which retain the overall "shape"of the molecule, but in which the electrophilicity at C-1 is much reduced by substituting a cyclopropane for the aziridine ring, to give a novel series called the cyclopropamitosenes, as illustrated below.

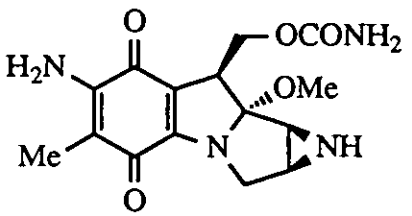
The objectives of this project have been (i) to design and synthesise novel

cyclopropamitosenes and analogues which hopefully enhance the antitumour activity but decrease cumulative myelosuppression,

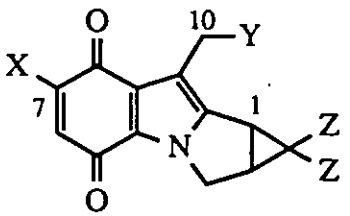
(ii) to synthesise analogues that are more specific for hypoxic cells rather than normal cells, preferentially at low drug concentration,

(iii) to determine if a correlation can be established between the electrochemical properties and the *in vitro* and / or *in vivo* activity of these novel cyclopropamitosenes and mitosenes, and

(iv) to investigate the mechanism of alkylation at the C-10 position of cyclopropamitosenes and mitosenes.



Mitomycin C

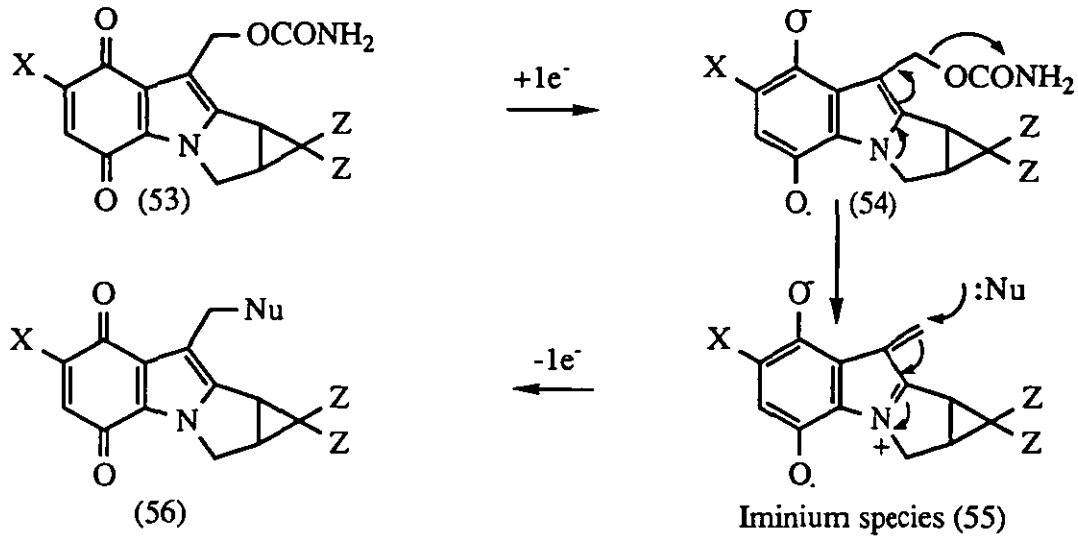


Cyclopropamitosenes (53)

The cyclopropamitosenes (53) are similar to mitomycin C except that (i) the aziridine has been replaced by a cyclopropane ring, and (ii) various substituents at the C-7 position. Since the aziridinomitosenes are considered to be the active species both *in vivo* and *in vitro* cyclopropamitosenes were synthesised. The numbering of these cyclopropamitosenes are similar to that of the trivial numbering of mitomycin C as illustrated above.

MMC on reductive activation has two electrophilic sites C-1 and C-10 as previously discussed. However in an attempt to investigate the role of C-10 in the alkylation process it was decided to decreased the electrophilicity at the C-1 position. Such compounds, cyclopropamitosenes (53) on reductive activation in the presence of a nucleophile, preferentially a biological one such as DNA, followed by oxidative work-up should lead to C-10 linked adducts (56) as shown in *Scheme 12*. These cyclopropamitosenes (53) in contrast to MMC have only one alkylation site, C-10; assuming that the cyclopropane ring remains intact.

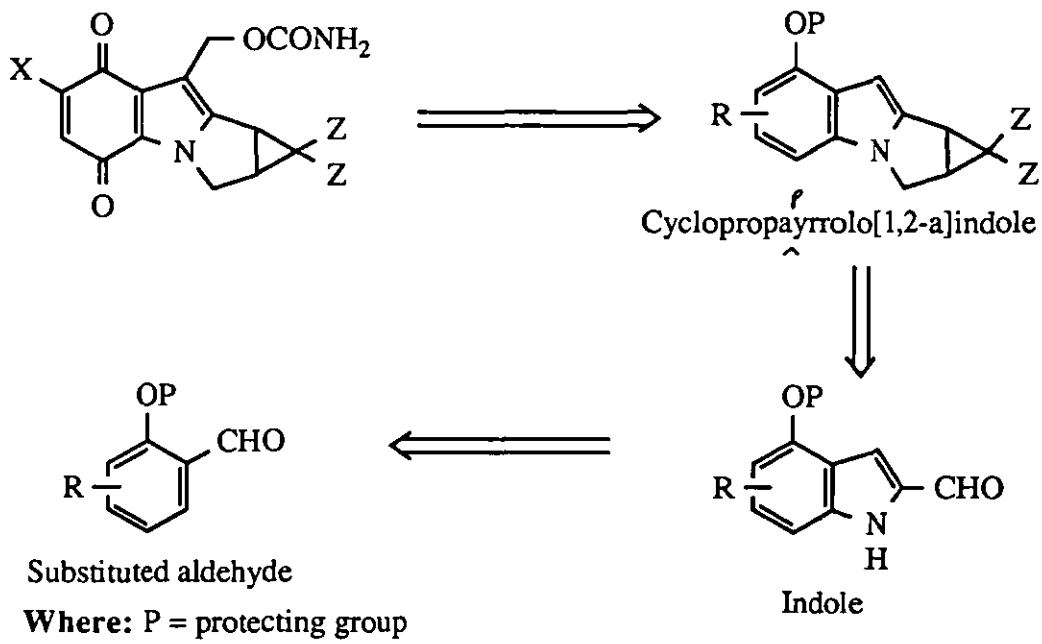
Scheme 12: Alkylation at C-10 of Cyclopropamitosenes Under Reductive Activation Conditions



Where: (1) X = OMe, Z = H (57)
(2) X = OMe, Z = Me (58)

The first consideration is a formal disconnection. Formal disconnections of (53) reasoned that this complex compound could be derived from substituted aldehydes using conventional transformations as illustrated in *Scheme 13.*

Scheme 13: Disconnection of Cyclopropamitosenes

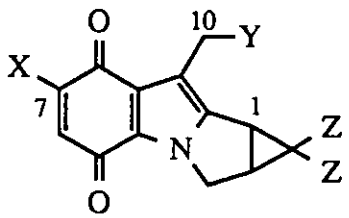


Where: P = protecting group

The methodology for the synthesis of cyclopropamitosenes (53) has been established by

Moody and Jones.[67, 68] The key step to the cyclopropapyrrolo[1,2-a]indole system is based on an intramolecular 1,3-dipolar cycloaddition of a diazo compound to an alkene double bond. The diazo compound was generated from the sodium salt of the tosylhydrazone, which was readily prepared from the appropriately substituted indole-2-carboxaldehyde as shown in *Scheme 13*. In the remainder part of the Chapter, the synthesis of various cyclopropamitosenes with different substituents for X, Y and Z will be discussed.

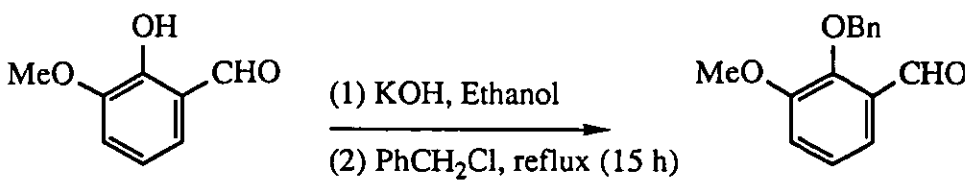
2.2. Synthesis of 9-Hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57)



Cyclopropamitosenes

Where: (57) X = OMe, Y = $OCONH_2$, Z = H
(58) X = OMe, Y = $OCONH_2$, Z = Me
(59) X = H, Y = OH, Z = H

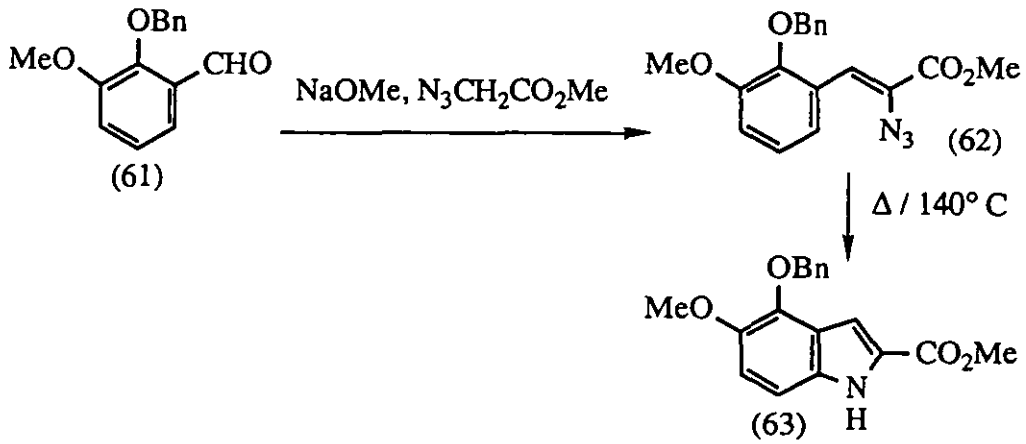
The A / B ring system was generated by azidocinnamate decomposition which was developed in the Hofmann laboratory in Imperial College.[69] *o*-Vanillin (60) was the ideal starting material being commercially available and reasonably cheap. It was necessary to protect the phenolic group of *o*-vanillin (60) so the functional hydroxyl group would not participate in any side reactions during the course of the synthetic route. Initially the alcohol was protected as a benzyl ether (61) by treatment of *o*-vanillin (60) with benzyl chloride in refluxing ethanol using potassium hydroxide as the base, in 62% yield.



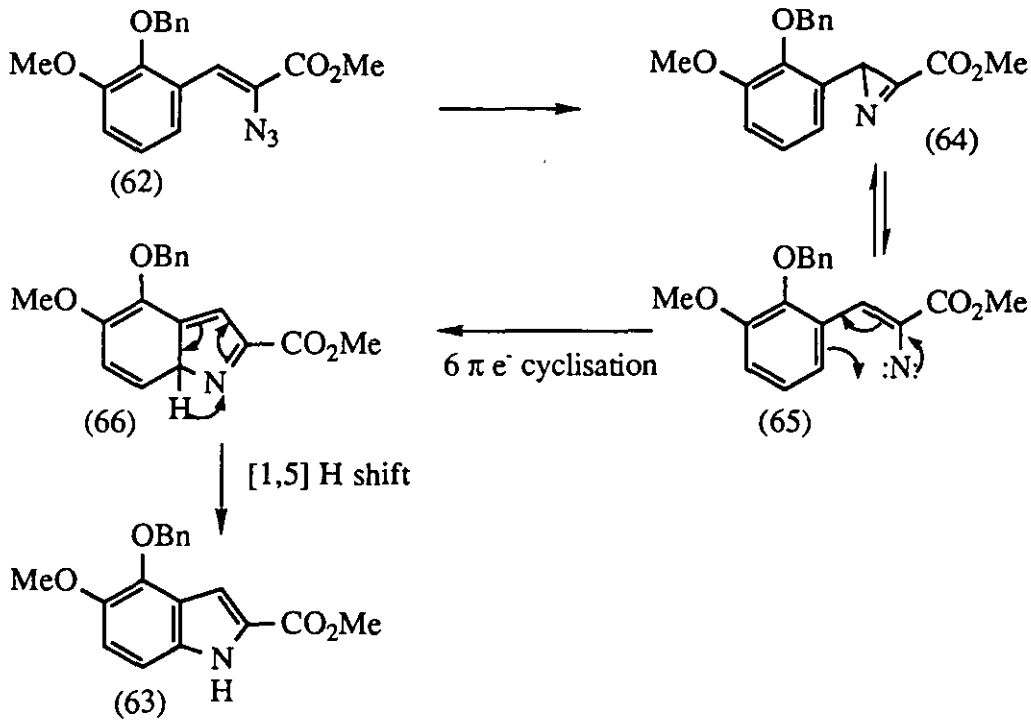
o-Vanillin (60) (61)

The protected phenolic aldehyde (61) was then condensed with methyl azidoacetate using sodium methoxide as a base in methanol, to afford a vinyl azide (62) in 82% yield. The resulting vinyl azide (62) was thermolysed, usually in refluxing xylene, to give the indole

ester (63) in typically good yield, 66%, as shown below.

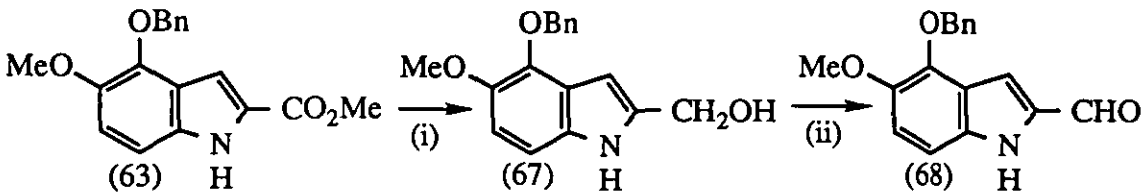


The mechanism of the indole formation is not a true nitrene 'insertion' into a C-H of the benzene ring but probably a 6 π electrocyclisation of the nitrene intermediate (65) to give (66) followed by a [1,5] H shift to afford the indole ester (63), as shown below. The geometry of the vinyl azide (62) is of no significance since the reaction proceeds *via* an 2H-azirine intermediate (64).



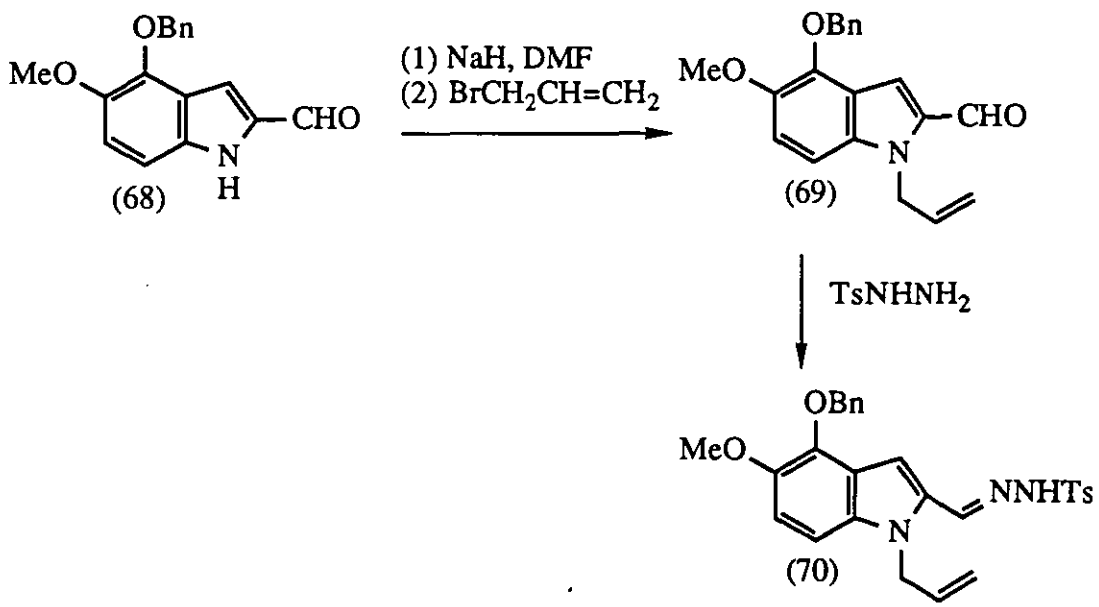
The substituted indole-2-carboxylate (63) was converted into the indole-2-carboxaldehyde (68) in two steps in excellent yield. The indole-2-carboxylate (63) was

reduced to the corresponding alcohol (67) with lithium aluminium hydride in refluxing THF, in 92% after 30 minutes. The alcohol (67) was oxidised to the indole-2-carboxaldehyde (68) by using standard conditions, manganese (IV) oxide, which is specific for allylic and benzylic hydroxyl groups under mild conditions. The indole-2-methanol (67) was stirred with a ten fold excess of manganese (IV) oxide in refluxing dichloromethane for 15 hours furnishing indole-2-carboxaldehyde (68) in 62% yield, as illustrated below.



Reagents: (i) Lithium aluminium hydride, refluxing THF; (ii) Manganese (IV) oxide, refluxing dichloromethane.

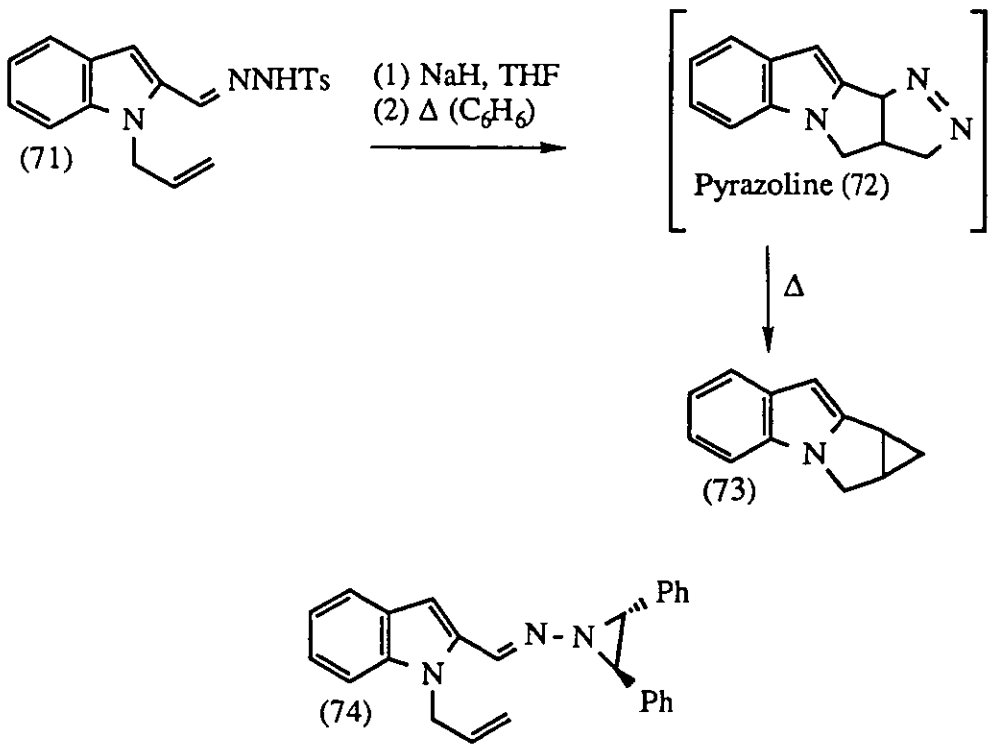
Now with the correctly substituted A / B ring system elaborated, it is necessary to expand the system to a tetracyclic compound. It was possible to allylate the indole nitrogen of indole-2-carboxaldehyde (68) by using sodium hydride in DMF, and then quenching with allyl bromide to afford (69) in excellent yield (99%). With the desired allylic group attached to the indole nucleus as in (69), it was converted into the tosylhydrazone (70) by condensation with tosylhydrazide in methanol at room temperature in 99% yield, as shown below.



The important key intramolecular [3+2] strategy was now applied. Literature precedent

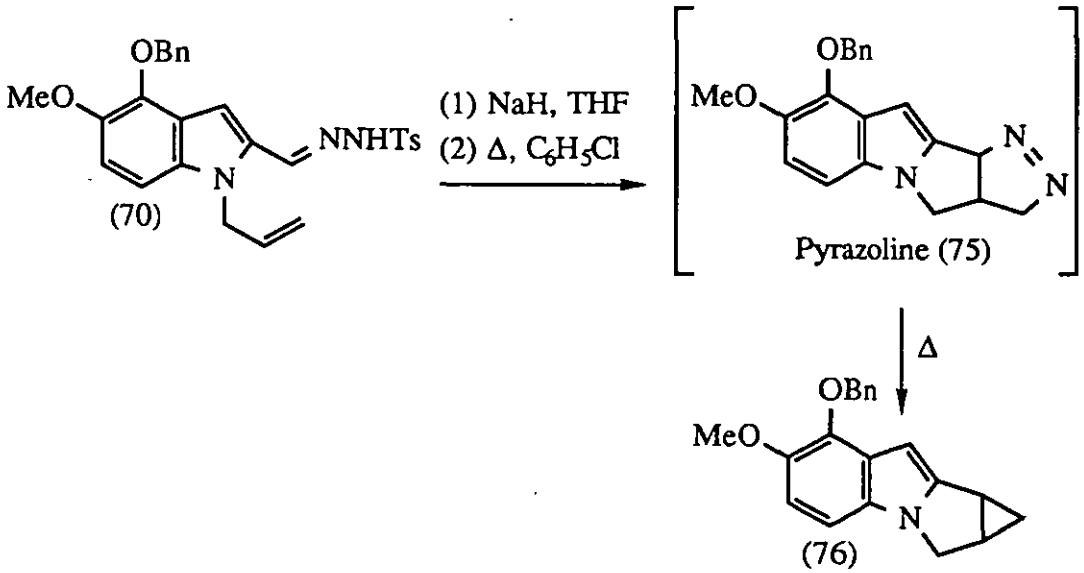
predicts that pyrazolines are formed, from 1,3-dipolar concerted pericyclic addition of a diazoalkane to an alkene.[70] The diazoalkane was generated by a variation of the Bamford-Stevens reaction,[71] which is thermolysis of the sodium salt of tosylhydrazone. Moody and Jones isolated the pyrazoline (72) by using tosylhydrazone (71) as the diazo precursor to generate the parent tetracyclic system (73) as shown in *Scheme 14*.[72] Formation of the sodium salt of (71) was achieved by treatment with sodium hydride in THF. The reaction mixtures were filtered and evaporated to dryness. The sodium salts were then thermolysed in refluxing benzene to afford the pyrazoline (72), in 29% yield. When the temperature was raised, the thermolysis of the pyrazoline (72) was achieved in refluxing xylene and the desired cyclopropapyrrolo[1,2-a]indole (73) was isolated in 89% yield, as shown in *Scheme 14*. It was possible to form pyrazoline (72) in somewhat higher yield, by using a different diazo precursor, the imine derived from *N*-amino-2,3-diphenyl aziridine (74). Thermolysis of (74) in boiling benzene gave (72) in 40% yield.[72]

Scheme 14

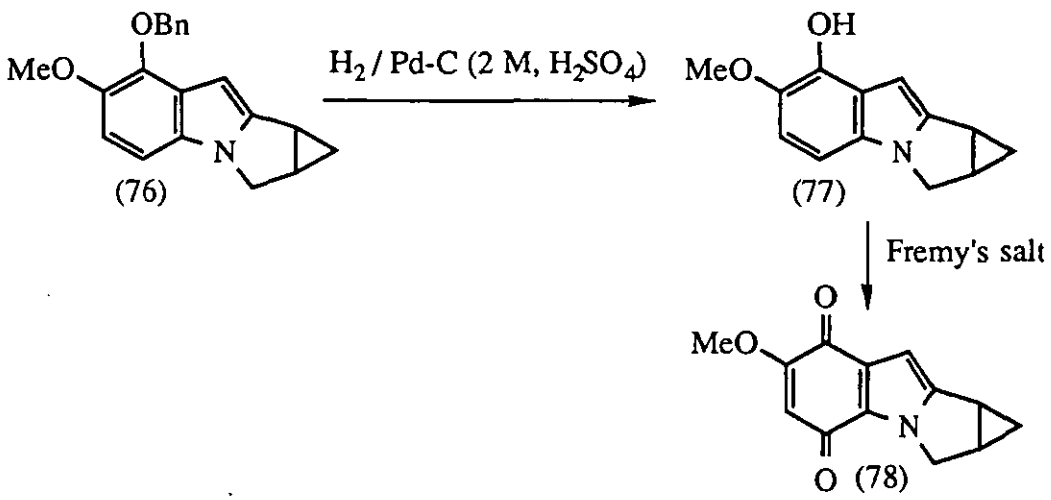


The above methodology was extended to tosylhydrazone (70). Formation of the sodium salt of tosylhydrazone (70) was achieved using sodium hydride in THF. In this case thermolysis was achieved in a higher boiling solvent, chlorobenzene. The desired cyclopropapyrrolo[1,2-a]indole (76) was formed in excellent yield, 94%, without isolating the presumed pyrazoline intermediate (75) as shown in *Scheme 15*.

Scheme 15



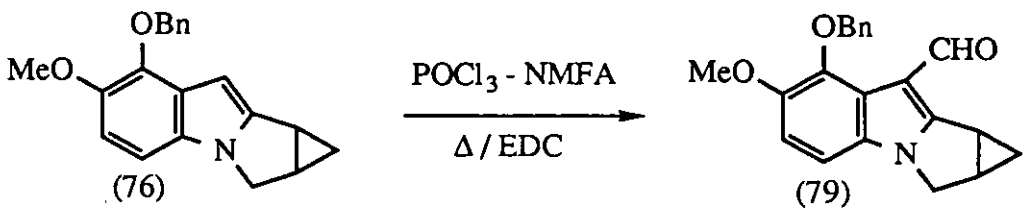
At this stage, the tetracyclic parent (76) has a choice of two alternative routes. Firstly, the cyclopropapyrrolo[1,2-a]indole (76) can be transformed into quinone (78) without the C-10 urethane group. Such a compound (78), can be used as a biological control. On reductive activation of cyclopropamitosene (78), as this molecule contains no alkylation sites *i.e.* C-10 or C-1, it should have no neoplastic activity, but this will be discussed in Chapter 5.



The *O*-benzyl group of the parent tetracyclic system (76) was removed by hydrogenolysis over palladium-charcoal (10%) in ethyl acetate and dilute sulphuric acid to give the corresponding phenol (77) in modest yield, 50%.[73] The phenol (77) was oxidised to the quinone (78) by Fremy's salt [74] in a mixture of water and acetone which was buffered with sodium dihydrogen phosphate affording the cyclopropamitosene (78),

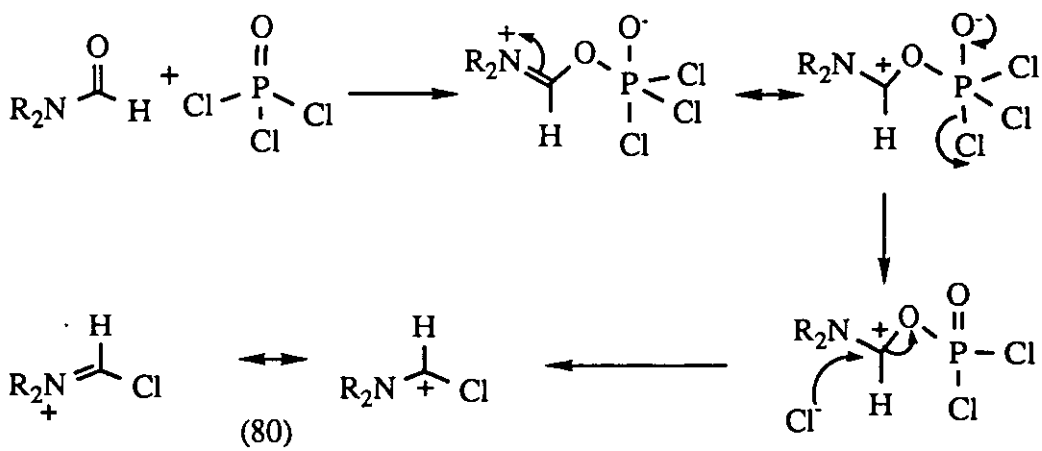
in 65% yield. Oxidation of the phenol to a quinone will be discussed later in this Chapter.

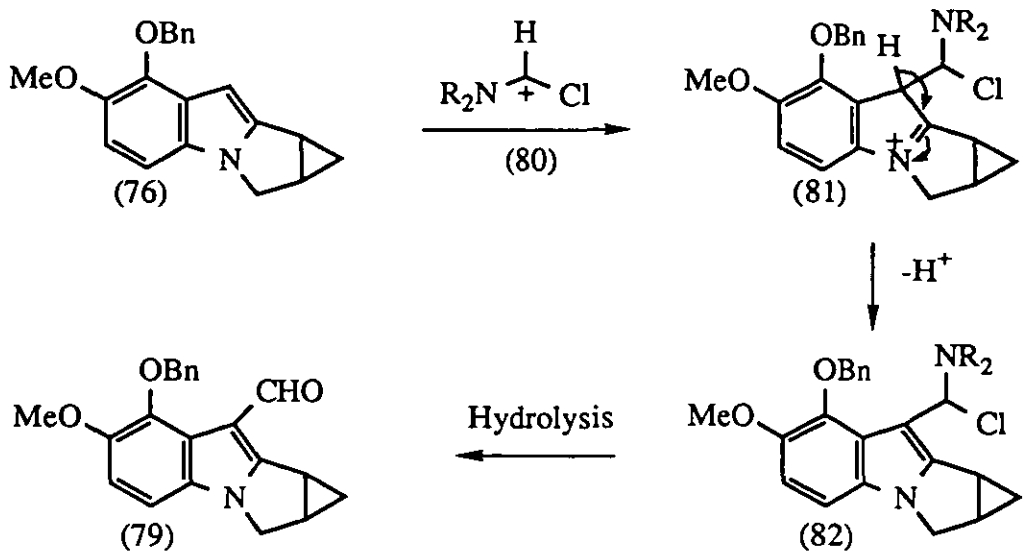
Secondly, the parent cyclopropapyrrolo[1,2-a]indole (76) can be transformed into a quinone with a C-10 urethane (57), *i.e.* a C-10 alkylation site. The parent tetracyclic has been synthesised and now it is necessary to bring in the last carbon unit, C-10. The C-10 unit was introduced by a variation of the Vilsmeier or Vilsmeier-Haack reaction, which is formylation of active aromatic rings (phenol and amines) and heterocycles using disubstituted formamides and phosphorus oxychloride. The cyclopropapyrrolo[1,2-a]indole (76) behaves as a simple indole, and it is possible to formylate the C-9 position. The parent cyclopropapyrrolo[1,2-a]indole (76) was stirred with a mixture of phosphorus oxychloride and *N*-methylformanilide (NMFA) in 1,2-dichloroethane to afford the C-9 formylated cyclopropapyrroloindole (79) in 76% yield.



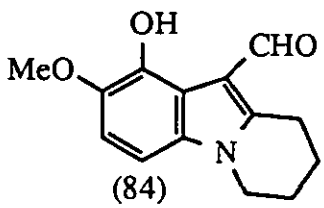
There is a considerable amount of controversy concerning the exact nature of the reactive species involved, but according to March [75] the attacking species is (80) and the mechanism is probably that shown in *Scheme 16*. Cyclopropapyrrolo[1,2-a]indole (76) undergoes electrophilic aromatic substitution at the C-9 position with intermediate (80) as the electrophile. The species (82) is very unstable and is easily hydrolysed with an aqueous solution of sodium acetate, to generate the formylated parent tetracyclic system (79).

Scheme 16

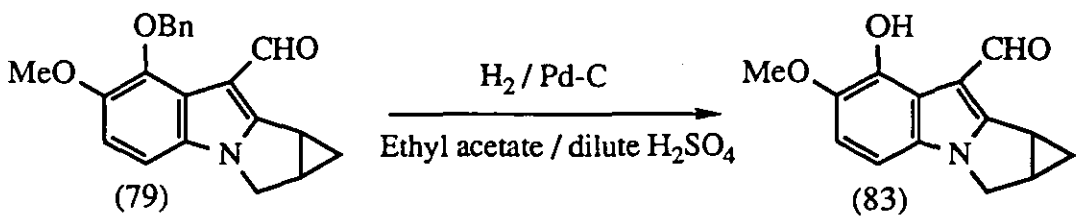




The *O*-benzyl of (79) should have been easy to remove by hydrogenolysis over palladium-carbon (10%) to give the corresponding phenol (83) but proved to be extremely difficult. During the course of the hydrogenolysis, not only was the *O*-benzyl group removed but also the cyclopropane ring was reduced giving (84). The R_f values of (84) and the desired product (83) were very similar, and the compounds were inseparable by chromatography.

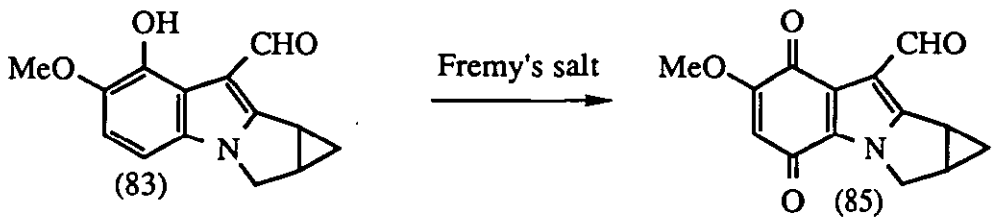


At this stage it was proposed that new reaction conditions were required. On addition of dilute sulphuric acid (2 M) and terminating the reaction before completion, it was possible to obtain pure material, in 79% yield as shown below.[73]



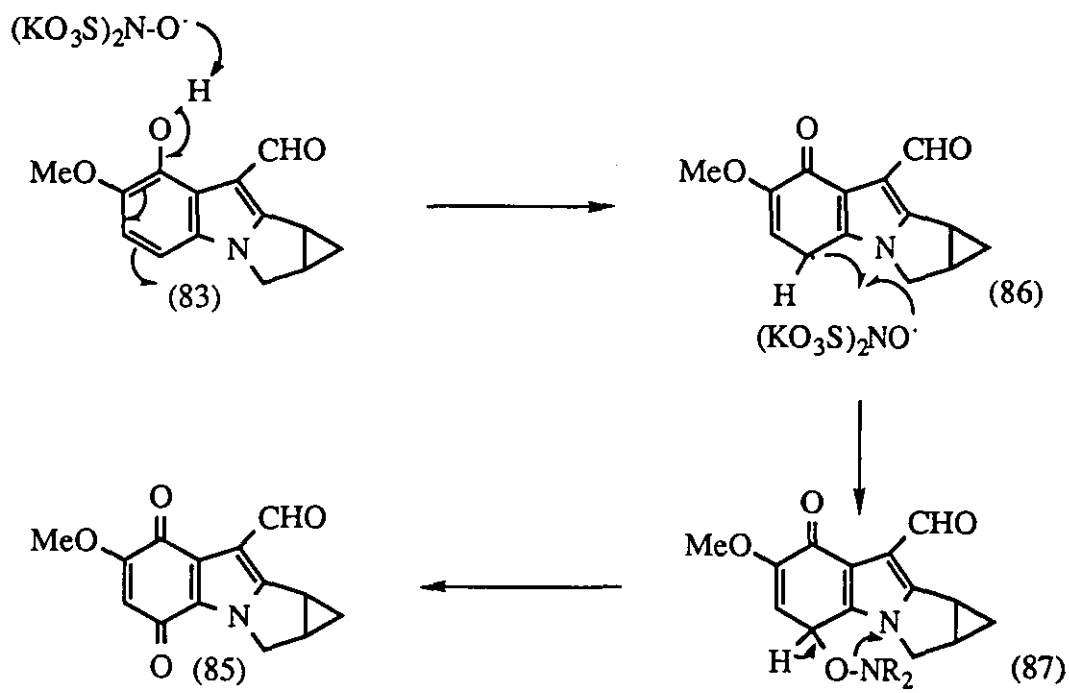
The next step was oxidisation of the phenol (83) to a quinone. *Ortho* and *para* diols or amino groups are easily oxidised to *ortho*- and *para*-quinones. Literature precedent

shows that this reaction has been successfully carried out with other groups para to the OH or NH_2: halogen, Me, OMe, and H.[74] A variety of oxidising agents, such as: acid dichromate, silver oxide, lead tetraacetate, cerium ammonium nitrate, and Fremy's salt (dipotassium nitrosodisulfonate-$(KSO_3)_2N$-O·) have been used. Fremy's salt is a particularly effective mild reagent, which is a stable free radical.

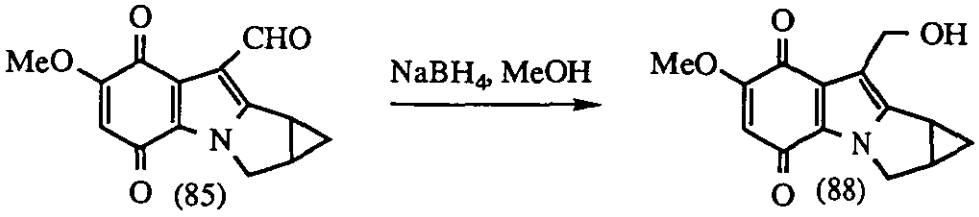


Phenol (83) was subsequent oxidised with Fremy's salt in a mixture of water and acetone which was buffered with sodium dihydrogen phosphate to give the tetracyclic quinone (85), in 95% yield. At this stage compound (85) is highly coloured, being a characteristic orange, unlike the indoles which are colourless solids. As already stated Fremy's salt is a stable free radical. The precise mechanism of oxidising phenols to quinones in not entirely clear but the sequence in *Scheme 17* is a likely mechanistic sequence, for the formation of compound (85).[76] Initially, $(KO_3S)_2N$-O· abstracts a hydogen atom from the phenol (83) generating an alkoxide radical which leads to radical (86). Radical (86) combines with the Fremy's salt to generate intermediate (87). The nitrogen-oxygen bond of $(KO_3S)_2N$-OR^1 (87) is very labile and $HN(SO_3K)_2$ is eliminated, generating the desired tetracyclic quinone (85) as illustrated below, in *Scheme 17.*

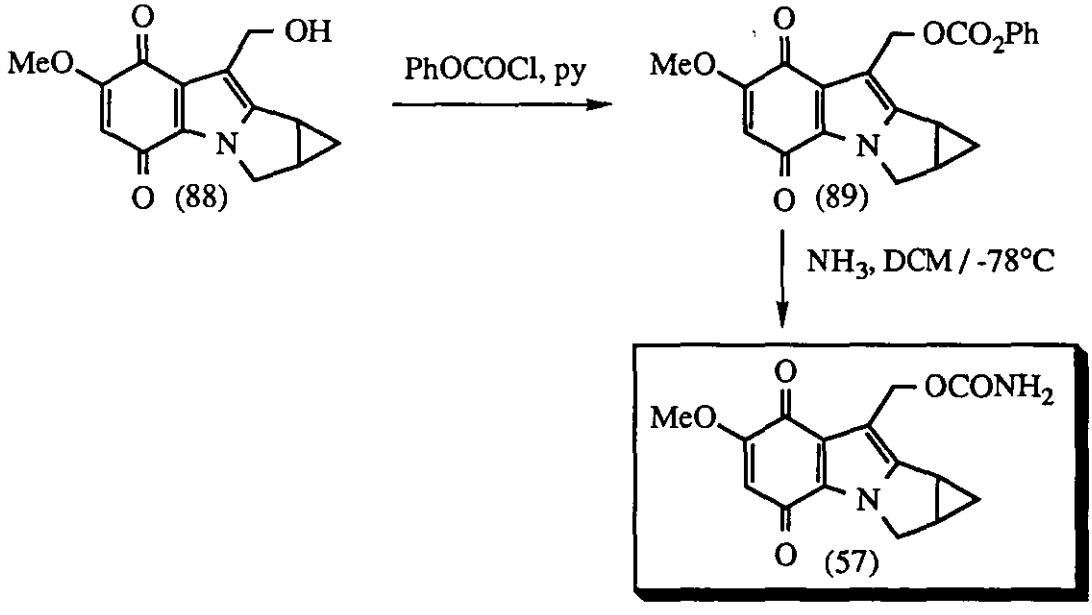
Scheme 17



Reduction of the tetracyclic system (85) was achieved by using sodium borohydride in methanol to give the desired quinone alcohol (88) in 90% yield. The quinone aldehyde (85) was initially reduced to the hydroquinone, accompanied by disappearance of the orange colour, which upon reoxidation by blowing air rapidly through the solution gave a red crystalline quinone alcohol (88).

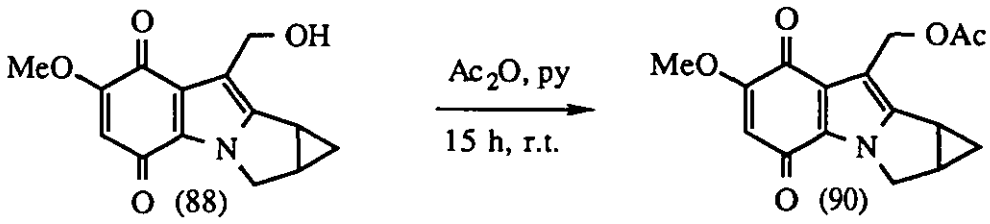


Transformation of the quinone alcohol (88) into the desired final quinone urethane (57) was achieved over two steps. Firstly, a phenyl carbonate (89) was formed by treating the alcohol (88) with phenyl chloroformate in pyridine under nitrogen for 2 hours. The phenyl carbonate (89) was isolated and purified by chromatography but it was not fully characterised. Once the phenyl carbonate (89) was generated, it was directly ammonolysed with ammonia in dichloromethane at -78°C to give the desired final product (57) in an overall yield of 83% for the two steps. The final urethane (57) was a brick red solid that was only sparingly soluble in dichloromethane, unlike all the previous quinones.



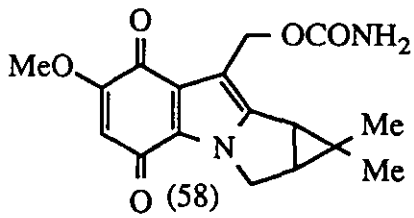
The desired novel cyclopropamitosene urethane (57) was synthesised in 14 steps from the commercially available *o*-vanillin in an overall yield of 7.5%. Despite its entirely

linear nature it was possible to furnish hundreds of milligrams of the final urethane (57). The quinone alcohol (88) was acetylated, by treatment with excess acetic anhydride in pyridine for 15 hours, in an excellent yield of 96%.



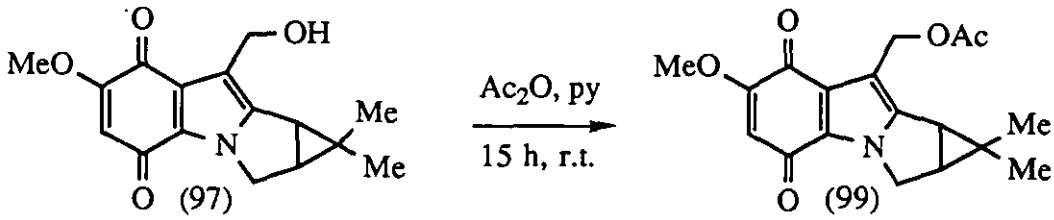
2.3. Synthesis of 9-Hydroxymethyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (58)

From the onset of this project one of the objectives was to synthesise analogues of the parent cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57), to investigate what happens to the antitumour activity when the molecule is structurally modified. In this case the two hydrogens at the C-1a site were substituted for two methyl groups as shown below. Again, this molecule only has one alkylation site, that is the C-10 position.



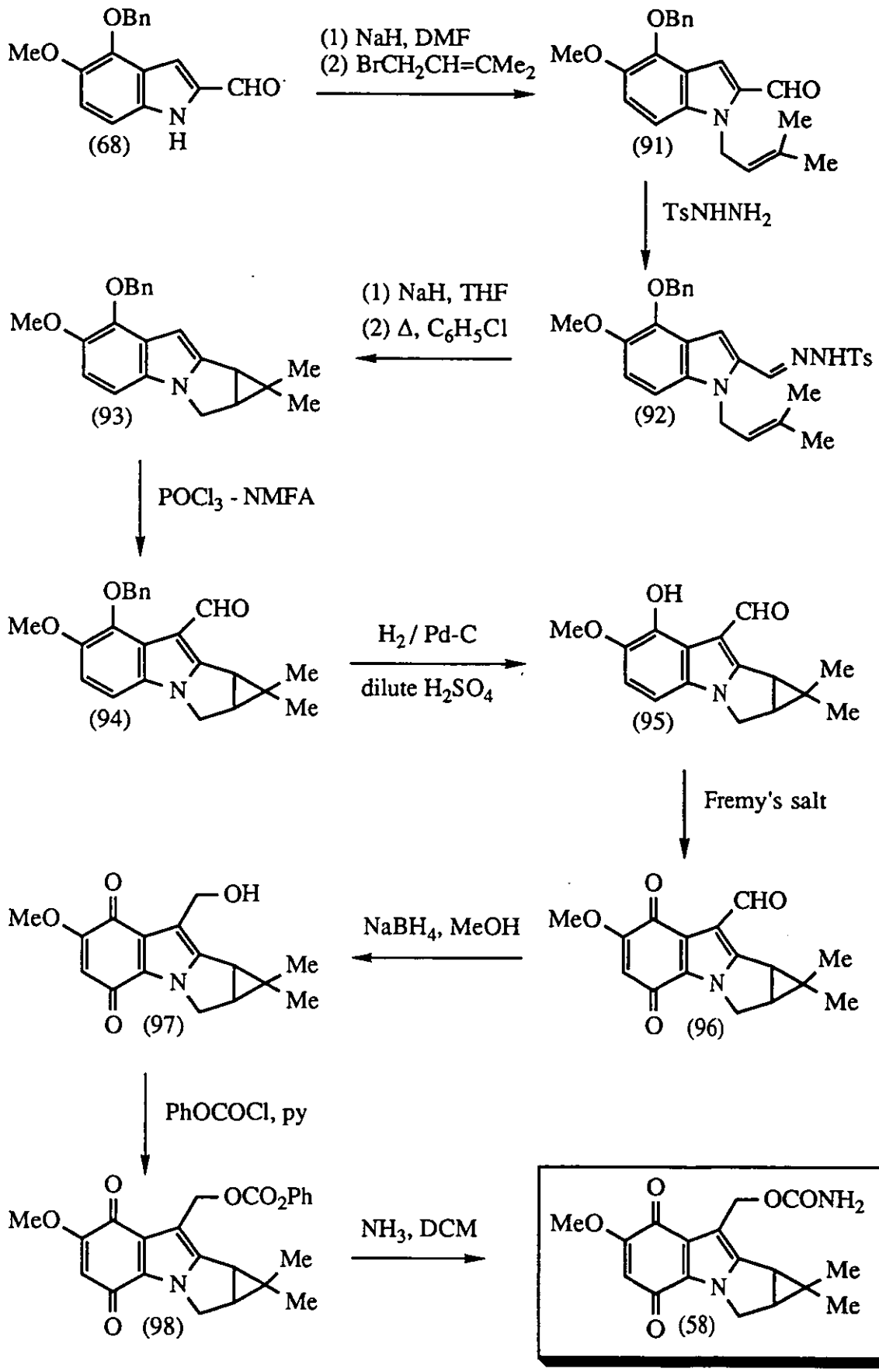
This synthetic route was again started from *o*-vanillin (60). The key difference between the the two synthetic routes was that this time the indole nitrogen of (68) was allylated with 4-bromo-2-methyl-2-butene. This was achieved by treating the indole aldehyde (68) with sodium hydride in THF, and then quenching with 4-bromo-2-methyl-2-butene to give the allylated indole nitrogen (91) in 65% yield, as shown in *Scheme 18*. This yield is not as good as the corresponding analogous compound (69) of the MeO series, which was obtained in 99%. The *N*-allylated indole (91) was converted into the tosylhydrazone (92) by condensation with tosylhydrazine, in 60-80% yield. Again, the 1,3-dipolar strategy of addition of a diazo compound to an alkene was applied. Formation of the sodium salt followed by thermolysis in chlorobenzene furnished the tetracyclic system (93) in a modest 60% yield. The cyclopropapyrrolo[1,2-a]indole (93)

was formylated as previously discussed to give (94) in 76% yield, identical to that of the corresponding analogous compound (79) of the MeO series. Debenzylation of (94) using hydrogenolysis over palladium-charcoal catalyst in ethyl acetate and dilute sulphuric acid formed the phenol (95) in 55% yield, which was considerably lower that the analogous compound (83) of the MeO series (79%). The phenol (95) was subsequently oxidised to the quinone (96) by Fremy's salt in 86%, that is comparable with the other similar series. The quinone aldehyde (96) was reduced to the alcohol (97) with sodium borohydride in methanol, in 69% yield. The alcohol (97) was then transformed into the final urethane (58) by ammonolysis of the phenyl carbonate (98) in DCM at -78°C, to afford (58) as an orange-red solid in 70% from (97) as shown on the next page in *Scheme 18*. This final urethane (58) was purified by column chromatography, and was soluble in dichloromethane, ethyl acetate and DMF. This again represents a 14 step synthesis of urethane (58), and despite its entirely linear nature was able to obtain milligrams of the final product (58), in an overall yield of 0.8%. This overall percentage yield is considerably lower than the corresponding MeO series.



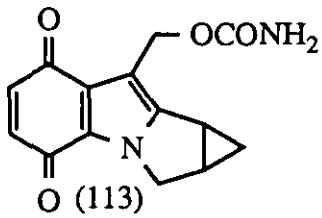
Again it was possible to acetylate the quinone alcohol (97), by treatment with an excess of acetic anhydride in pyridine to afford (99) in 66% yield. This yield is 30% lower than the corresponding analogous compound (90) of the MeO series. The biological and electrochemical properties of (58) and (99) will be discussed in Chapter 5. Compound (58), like the final urethane (57) was subjected to C-7, exchange reactions which will be discussed in Chapter 3.

Scheme 18


OBn
MeO
CHO
N
H
(68)
(1) NaH, DMF
(2) BrCH2CH=CMe2
Me
Me
(91)
TsNHNH2
NNHTs
(92)
(1) NaH, THF
(2) Δ, C6H5Cl
(93)
POCl3 - NMFA
(94)
H2 / Pd-C
dilute H2SO4
OH
(95)
Fremy's salt
O
(96)
NaBH4, MeOH
(97)
PhOCOCl, py
OCO2Ph
(98)
NH3, DCM
OCONH2
(58)

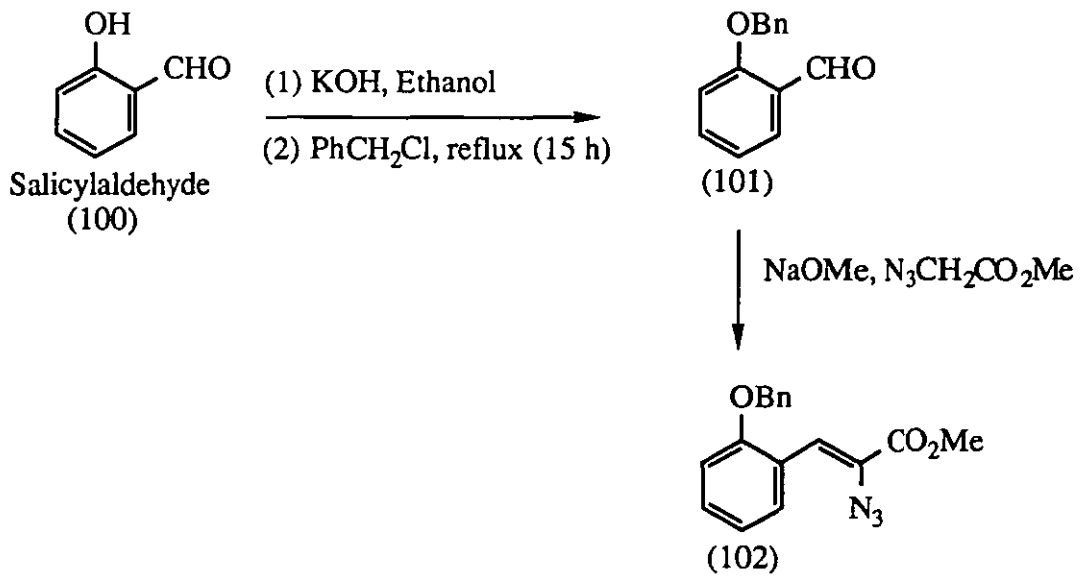
2.4. Synthesis of 9-Hydroxymethyl-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (59)

The rationale for the design of various other cyclopropamitosenes was extended to varying substituents at C-7. Initially, two hydrogens were replaced by two methyl groups at the C-1a positions, and the next logical step would be to investigate the effect on antitumour activity of cyclopropamitosenes without the C-7 methoxy group. Such a compound (113), would only have one alkylation site, the C-10 position but it would indicate if the C-7 methoxy group was necessary for biological activity. All attempts to produce to the final urethane (113) proved to be fruitless. It was possible however to obtain the quinone alcohol (59), and the synthesis of this alcohol (59) is now described.



(113)

This time the synthetic route started from the commercially available chemical, salicylaldehyde (100). The salicylaldehyde (100) was protected as a benzyl ether to afford (101) in 64% yield. The appropriately protected aldehyde (101) was condensed with methyl azidoacetate in methanol, using sodium methoxide as a base to give the vinyl azide (102) in 82% yield. The resulting vinyl azide (102) was thermolysed in refluxing xylene, yielding an indole ester (103) in 80% yield.

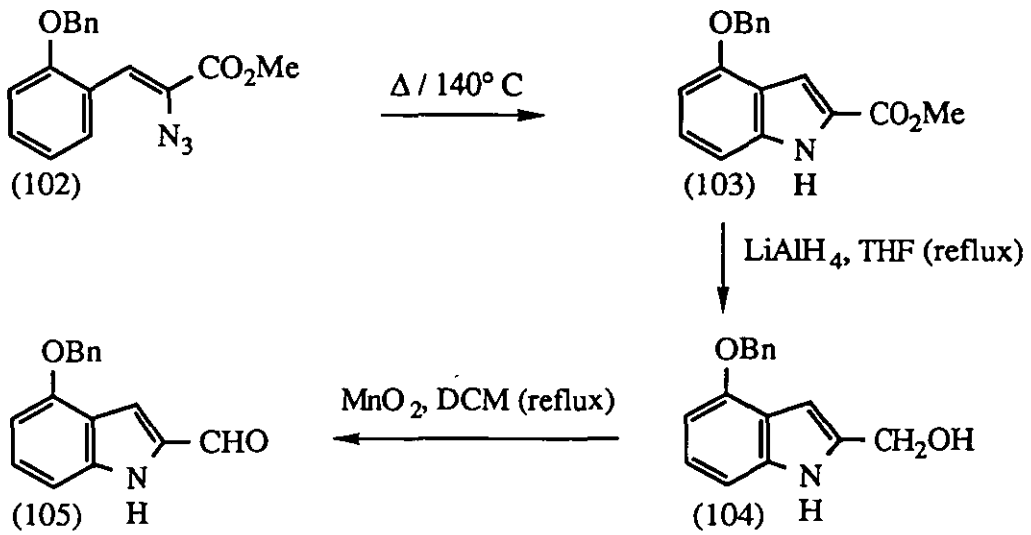


Salicylaldehyde (100)

(101)

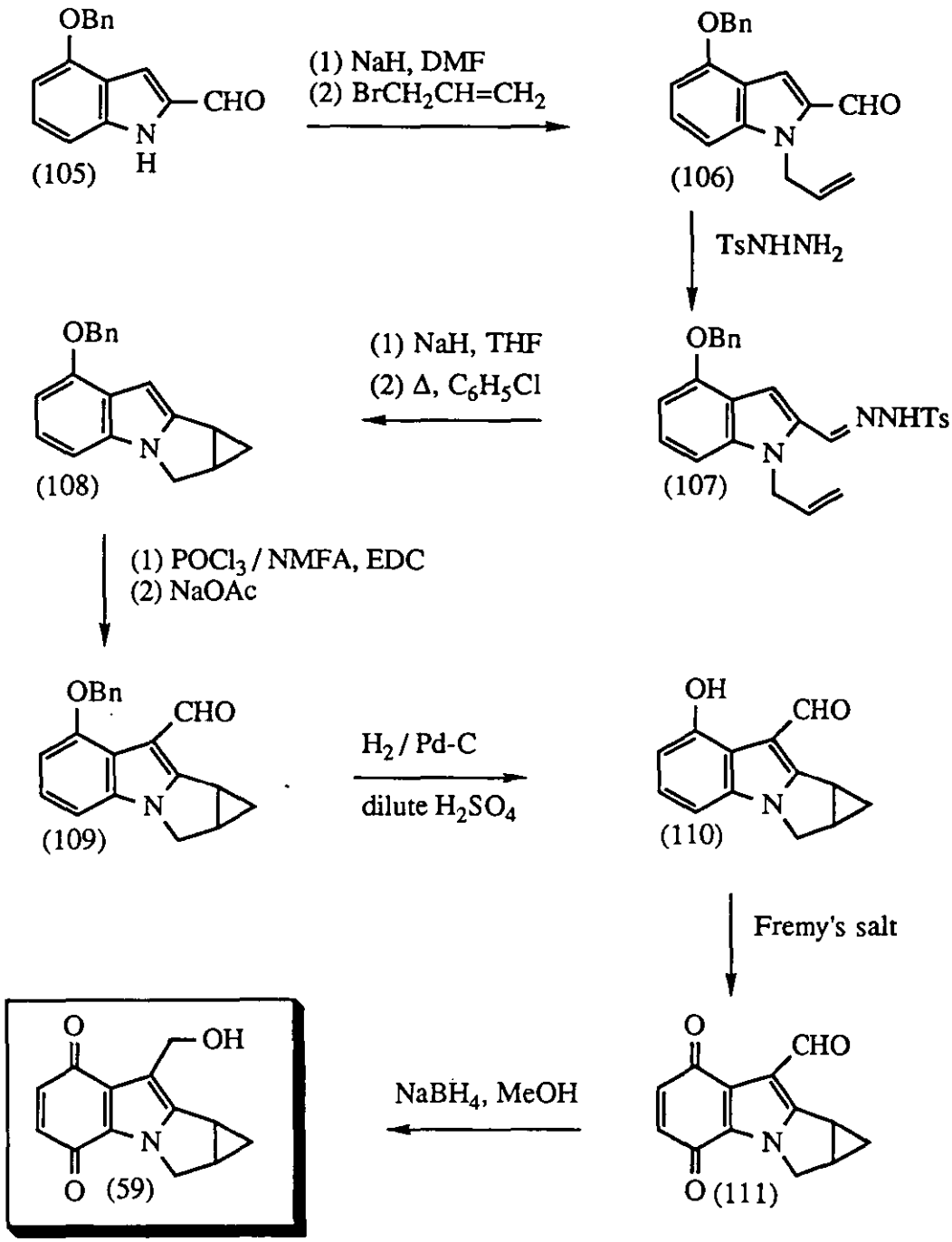
(102)

The indole ester (103) was converted into the indole aldehyde (105) in two steps: firstly reduction of the indole ester (103) with lithium aluminum hydride in refluxing THF giving (104) (88%), and oxidisation of the indole alcohol (104) with manganese (IV) dioxide in refluxing dichloromethane affording (105) in an excellent yield of 86%.

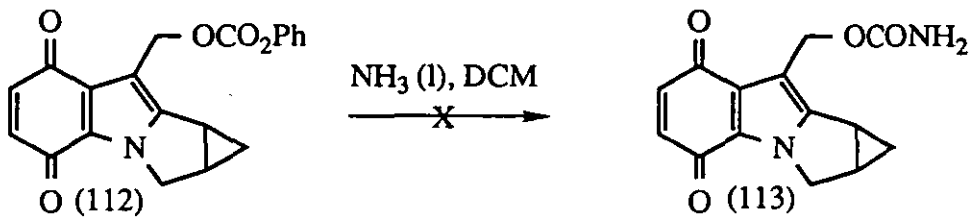


It was possible to *N*-allylate the indole aldehyde (105), by treatment with sodium hydride and then quenching with allyl bromide which formed the *N*-allylated indole (106) in 99% yield. Its tosylhydrazone (107) was formed by condensation with tosylhydrazine in methanol, in a good yield of 87%. Formation of the salt of the tosylhydrazone (107) was again achieved by treatment with sodium hydride in THF, thermolysis of which, in chlorobenzene gave the parent tetracyclic system (108) directly in an excellent yield, 96%. Formylation of the parent tetracyclic system (108) was achieved using standard Vilsmeier chemistry; phosphorus oxychloride and *N*-methylformanilide in 1,2-dichloroethane, to give (109) in 89% yield. The formylated tetracyclic system (109) was transformed into the quinone alcohol (59) in the following steps. Debenzylation of (109) using hydrogenolysis over palladium-charcoal catalyst (10%) in ethyl acetate and a few drops of dilute sulphuric acid formed phenol (110) (68%). Oxidation of the phenol (110) using Fremy's salt resulted in a *p*-quinone (111) (64%). Reduction of the quinone aldehyde (111) using sodium borohydride in methanol under the same conditions as previously stated (stirring with sodium borohydride in methanol under nitrogen for 4 hours) failed to give the desired quinone alcohol (59) but baseline material instead. However, on reducing the reaction time from 4 hours to 25 minutes, the desired quinone alcohol (59) was obtained in an excellent yield of 89% yield. The quinone alcohol (59) formed was not stable in the presence of sodium borohydride for a long period of time and just decomposed. All these reactions are summarised below in *Scheme 19*.

Scheme 19



Transformation of the quinone alcohol (59) into the required final urethane (113) proved to be fruitless. The alcohol (59) was converted to the phenyl carbonate (112), using phenyl chloroformate in pyridine. Ammonolysis of the phenyl carbonate (112) in dichloromethane with either (i) ammonia gas at -78°C, or (ii) 0.88 NH_3 did not afford the desired urethane (113) but baseline material or phenyl carbonate respectively.



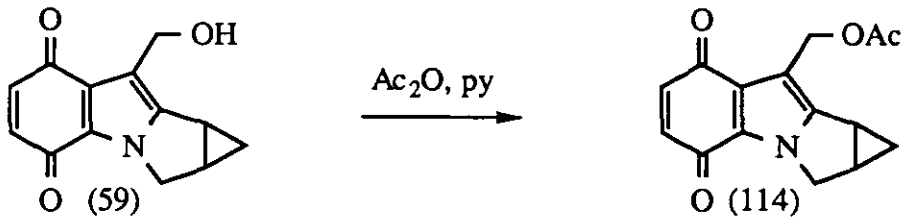
Alternative routes from the quinone alcohol (59) to the final urethane were also attempted. Initially, the alcohol (59) was treated with sodium cyanate (2 mole equivalents) in benzene. The suspension was stirred slowly while trifluoroacetic acid (2.1 mole equivalents) was added rapidly. The temperature was raised to 37°C and the mixture was then cooled in an ice bath for 5 minutes. The mixture was stirred slowly at room temperature for 15 hours.[77] Alternatively, the quinone alcohol (59) was treated with aqueous potassium isocyanate at 75°C for 10 minutes, and only baseline material was obtained.[78] In a last attempt to get this extremely difficult reaction to give the desired final urethane (113), we decided to monitor the reaction by ^{1}H NMR (250 MHz). The alcohol (59) was treated with an excess of trimethylsilyl isocyanate in $CDCl_3$ and a proton spectra was run every 30 minutes for 2 hours. From the spectra, only starting material was present. On addition of triethylamine after this period of time, the molecule decomposed to baseline material again.[79] These different reaction conditions are summarised in *Table 5*.

Table 5

Reactant	Reaction Conditions	Products of Reaction
(i) Phenyl carbonate (112)	NH_3, DCM @-78°C (15 min)	Baseline material
(ii) Phenyl carbonate (112)	0.88 NH_3 (l), DCM r.t. (15 h)	Starting material
(iii) Quinone alcohol (59)	(a) NaOCN, C_6H_6	Starting material with
	(b) CF_3CO_2H, r.t. (15 h)	Baseline material
(iv) Quinone alcohol (59)	(a) THF / H_2O	Baseline material
	(b) KNCO @ 75°C (10 min)	
(v) Quinone alcohol (59)	*^{1}H NMR Experiment*	
	(a) TMSNCO, $CDCl_3$	Baseline material
	(b) Et_3N	

Note: The phenyl carbonate (112) was not characterised but monitored by TLC

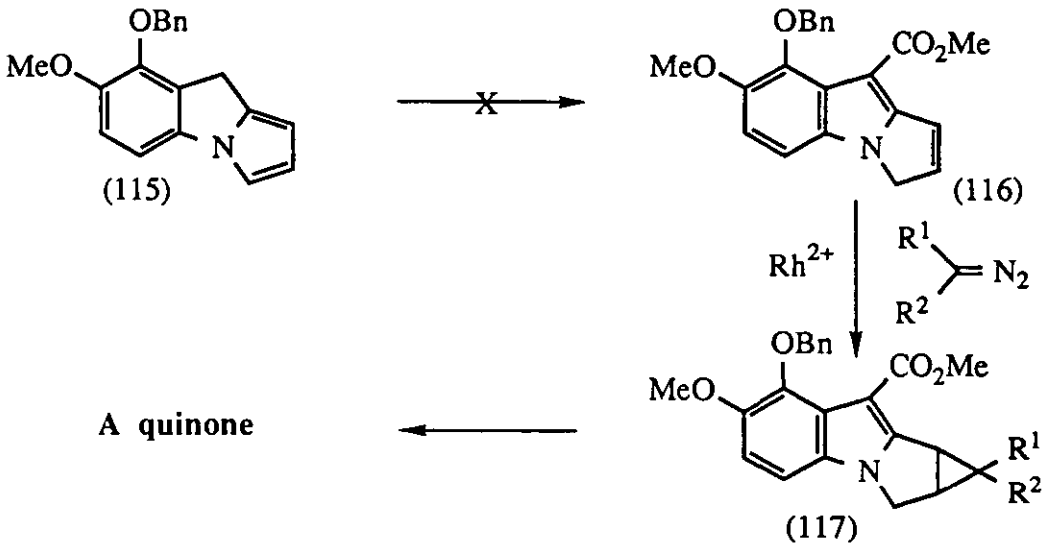
In an attempt to further our investigation of the chemistry of the quinone alcohol (59), it was decided to prepare the acetyl derivative (114), in 56% yield. The quinone alcohol (59) was treated with an excess of acetic anhydride in pyridine, which was stirred for 15 hours at room temperature. The desired product (114) was obtained but unfortunately in small quantities. Unfortunately, it was not considered useful at this stage to repeat the entire sequence again especially as the final urethane (113) could not be synthesised, due to lack of time.



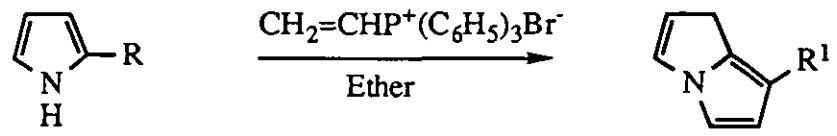
It was likely that the desired final urethane (113) was not isolated because the compound was too unstable under the reaction conditions attempted. The 7-methoxy group of the final urethanes (57) and (58) appears to stabilise these compounds, maybe by an inductive effect. It is quite remarkable how such a small structural change at the C-7 position can totally alter the reactivity of the quinone alcohols and this may be very important for antitumour activity. It would be interesting to extend the study of this series (7-H series) further as it might enlighten chemists as to what functional groups at position 7 stabilise the final urethane and enhance the neoplastic activity, so that better analogues can be synthesised.

2.5. *Synthesis of Mitosenes*

The next logical extension of this work would be (i) to synthesise unsaturated 3H-pyrrolo[1,2-a]indoles (116); such compounds in the presence of a diazo compound and a chiral rhodium catalyst should lead to chiral cyclopropapyrrolo[1,2-a]indoles (117) as shown below, and (ii) to synthesise saturated 3H-pyrrolo[1,2-a]indoles without the cyclopropane ring. It was hoped that the biological activity of the mitosenes after oxidation could be determined. This will be discussed in Chapter 5.



For both routes it was necessary to synthesise 9H-pyrrolo[1,2-a]indole (115). Previous work reported by authors such as Schweizer and Kenneth,[80] Hirata, Yamada and Matsui,[81, 82] and Franck and Bernady [83] has described the synthesis of 9H-pyrrolo[1,2-a]indole by means of the general ring synthesis using vinyltriphenylphosphonium bromide. Appropriately substituted pyrrole-2-aldehydes (118) and indole-2-aldehydes (120) were treated with sodium hydride in either diethyl ether or THF, and then quenched with vinyltriphenylphosphonium bromide to afford the desired 9H-pyrrolizine products (119) and 9H-pyrrolo[1,2-a]indole (121) in generally good to excellent yield as shown below.

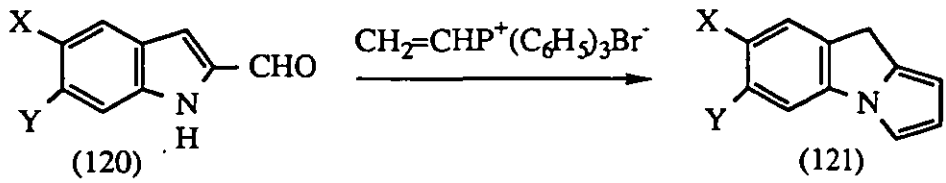


(118)

Where: R = CHO
= C(O)Me

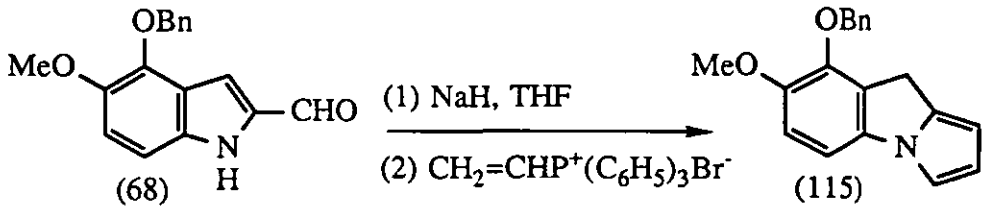
(119)

Where: R^1 = H (87%)
= Me (43%)

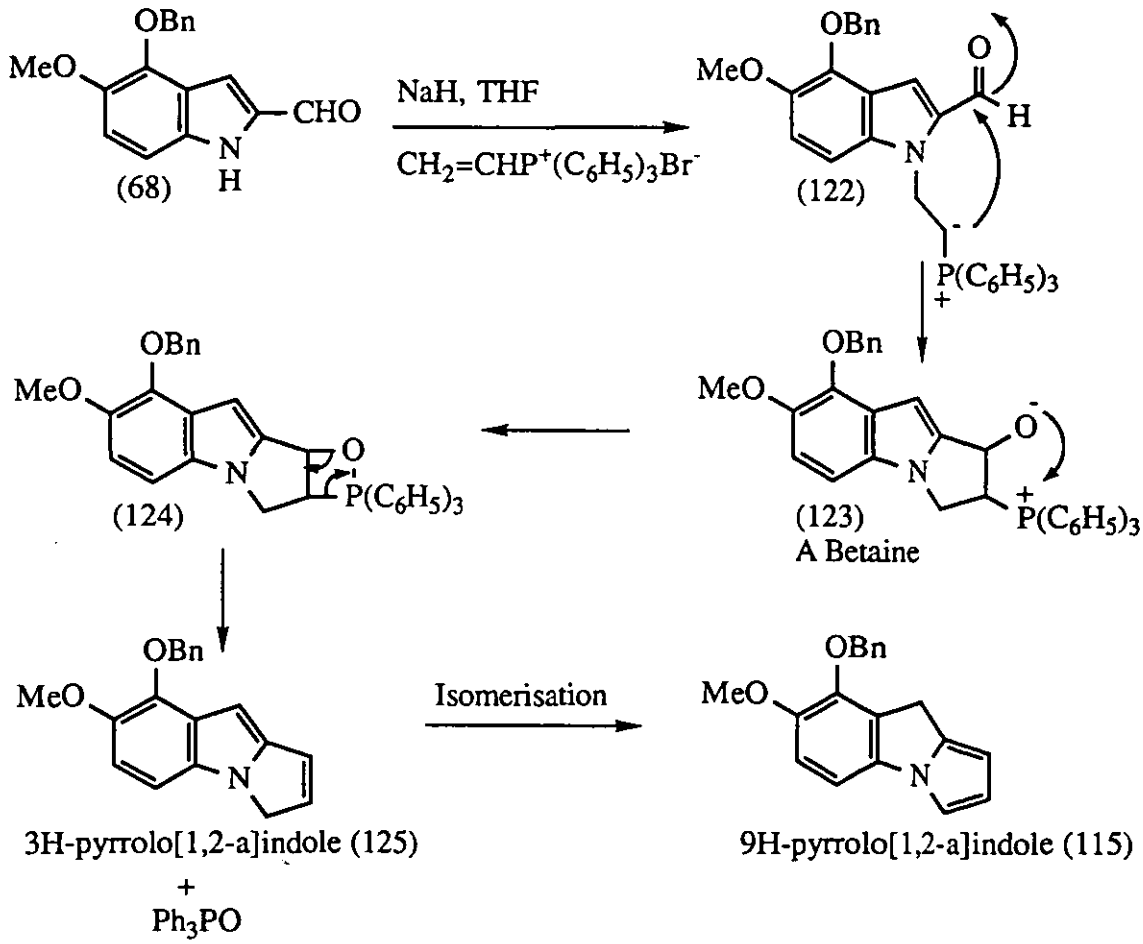


Where: X = H / OMe / OBn
Y = H / Me

This idea was extended to indole-2-carboxaldehyde (68) which had previously been synthesised. Indole-2-carboxyaldehyde (68) was subjected to a modified Wittig reaction, the probable mechanism of which is illustrated below.[84]



Scheme 20: Mechanism for the Formation of the 9H-Pyrrolo[1,2-a]indole



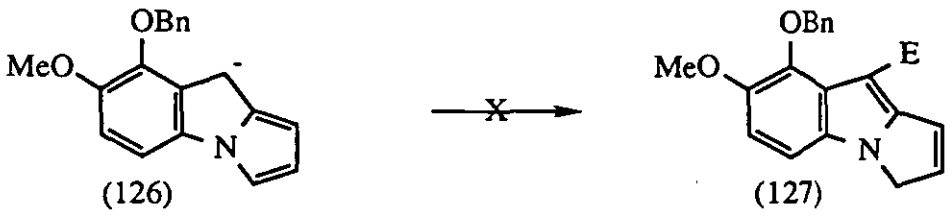
The indole-2-carboxaldehyde (68) was treated with sodium hydride in THF to generate an anion. The anion was then quenched with triphenylphosphonium bromide in refluxing THF for 15 hours under nitrogen to generate a new phosphorus ylide (122). The phosphorus ylide (122) then attacked the aldehyde group affording a dipolar betaine (123). The betaine intermediate (123) in the Wittig reaction is unstable and decomposes at temperatures above 0°C, to yield 3H-pyrrolo[1,2-a]indole (125) and triphenylphosphine oxide, as shown in *Scheme 20*. From previous studies by Frank and Bernady [83] on the chemistry of pyrrolo[1,2-a]indole it was concluded that the 3H product is less stable than the 9H isomer. This is also the case with our pyrrolo[1,2-a]indole. Initially, the 3H-pyrrolo[1,2-a]indole was formed by the Wittig reaction which then isomerises to give the more stable 9H isomer (115), in 93% yield, as shown below in *Scheme 20*.

The 9H-pyrrolo[1,2-a]indole anions have been acylated by a group of electrophiles, including dimethyl carbonate, ethyl chloroformate, dimethyl oxalate, phenyl isocyanate, and carbon dioxide.[81, 82, 83] Unfortunately, in our hands with a different 9H-pyrrolo[1,2-a]indole (115) all attempts at acylation proved to be fruitless, these are now discussed. The acylation of 9H-pyrrolo[1,2-a]indole (115) using potassium *tert*-butoxide / dimethyl carbonate in either THF of diethyl ether did not afford the key intermediate, acylated 3H-pyrrolo[1,2-a] indole (127). Alternatively, using a stronger base such as *n*-butyllithium, mainly starting material was isolated. The use of a stronger nucleophile such as methyl chloroformate yielded a mixture of starting material and baseline. All these different conditions are summarised and shown in *Table 6*.

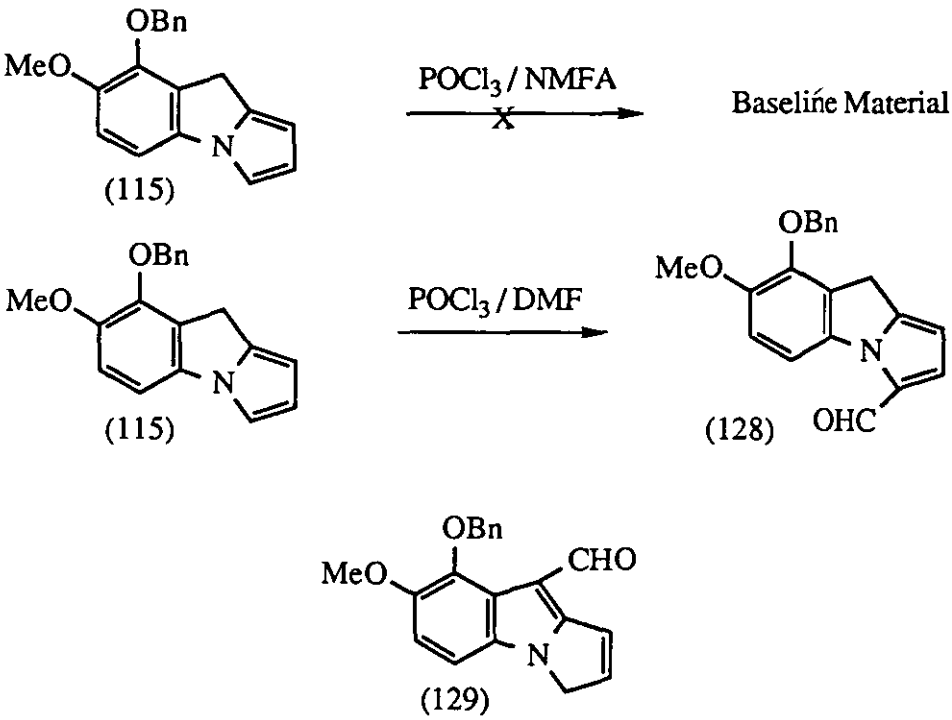
Table 6

Reactant	Reaction Conditions	Desired Product	Products Obtained
(115)	(i) $Me_3CO^-K^+$, THF (ii) $(MeO)_2CO$, reflux (15 h)	(127)	SM and Baseline
(115)	(i) $Me_3CO^-K^+$, Ether (ii) $(MeO)_2CO$, reflux (15 h)	(127)	SM
(115)	(i) $Me_3CO^-K^+$, THF (ii) $ClCO_2Me$, r.t. (15 h)	(127)	SM and Baseline
(115)	(i) *n*-BuLi, Ether @-78°C (ii) $(MeO)_2CO$, 0°C - r.t. (4 h)	(127)	SM

SM: Starting material



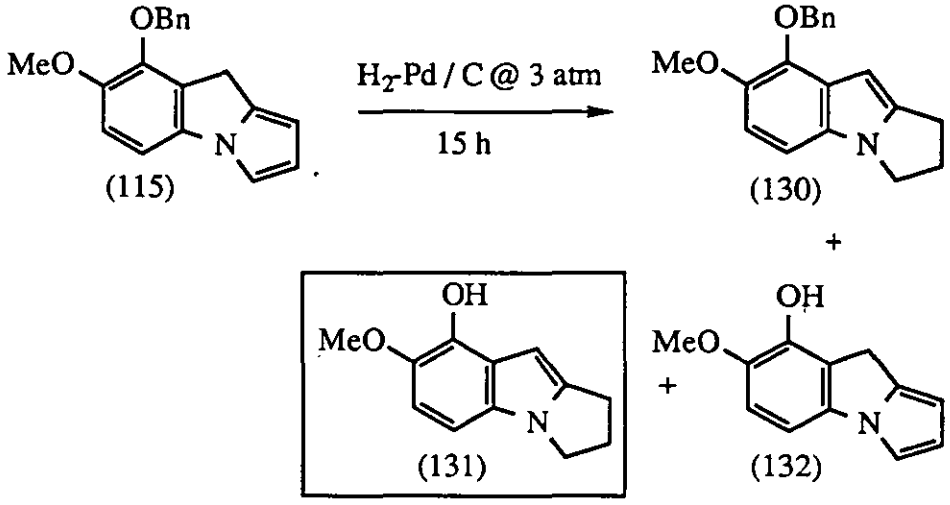
As all the acylation reactions proved to be unsuccessful, we decided to formylate 9H-pyrrolo[1,2-a]indole (115) at the C-9 position. Initially, formylation of 9H-pyrrolo[1,2-a]indole (115) was attempted under exactly the same conditions as previously discussed, using *N*-methylformanilide, and phosphorus oxychloride in refluxing 1,2-dichloroethane for 1.25 hours to give mainly baseline material. However, by altering the reaction conditions to DMF-phosphorus oxychloride,[85] the desired product (129) was not formed, instead (128) was obtained in low yield, 38%, as shown below. The structure of compound (128) was assigned by ^{1}H NMR (400 MHz) and nOe experiments.



At this stage as all the acylation and formylation reactions failed to give the desired unsaturated 3H-pyrrolo[1,2-a]indole (127) it was decided to concentrate our efforts on the second aim of this part of the project, which was to synthesise a saturated 3H-pyrrolo[1,2-a]indole. To do this it was necessary to investigate the chemistry of the tricyclic indole system (115). Hydrogenation in the presence of palladium-charcoal

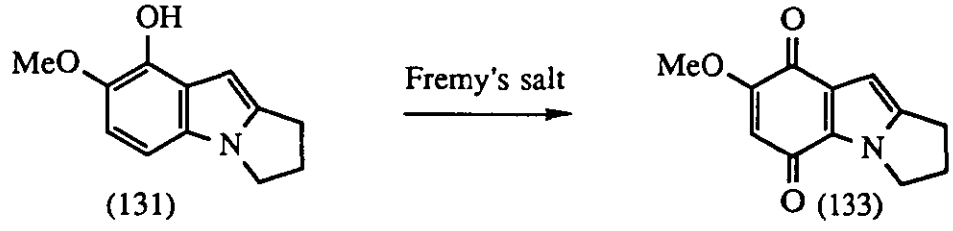
catalyst (10%) in ethyl acetate was attempted initially. The aim of this experiment was to obtain compound (130). However, after 15 hours at three atmospheres of hydrogen, mainly unreacted starting material was recovered and compounds (130), (131), and (132) were obtained as minor products, as shown in *Scheme 21*.

Scheme 21



These were all characterised by ^{1}H NMR (250 MHz). However, when the reaction time for hydrogenation at 3 atmospheres pressure was increased to 7 days, the major product obtained was (131) (52%). Smaller amounts of (130) (6%) and (132) (3%) were also isolated. This reaction gave product (131) by removal of the *O*-benzyl group followed by reduction of one of the double bonds of the pyrrole ring. Since the aromaticity of the pyrrole ring is now destroyed, the remaining double bond isomerises to generate a new stable 3H-pyrrolo[1,2-a]indole (131).

This 3H-pyrrolo[1,2-a]indole (131) is useful in two respects. Firstly, analogues can be synthesised without the C-10 urethane substituent, as shown below. 3H-pyrrolo[1,2-a]indole (131) as previously discussed was oxidised to the quinone (133) using Fremy's salt, in a modest yield, 57%.[74]

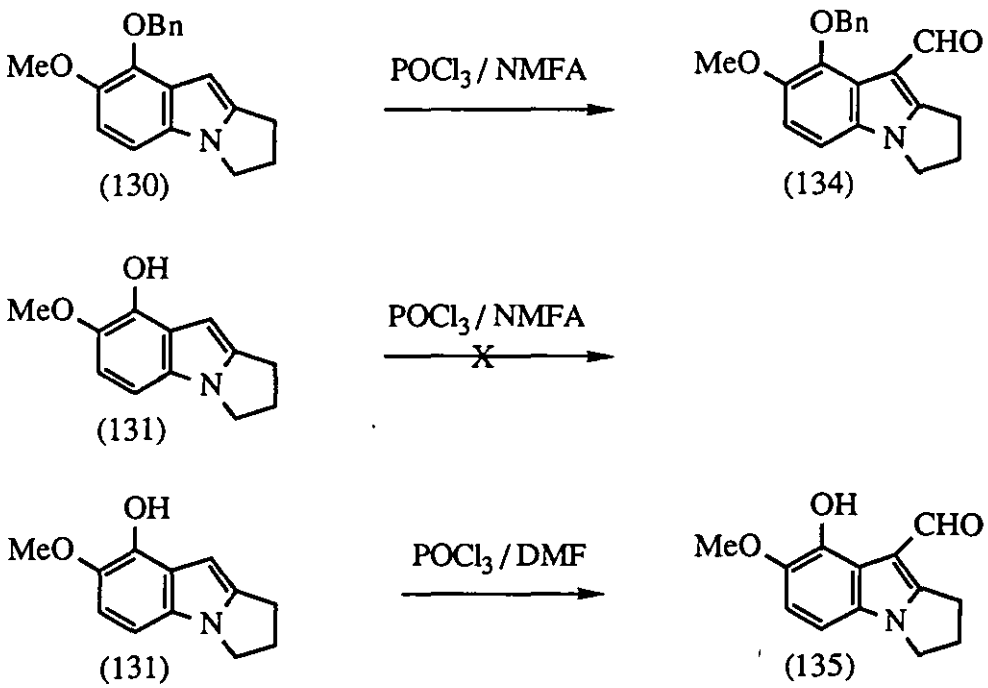


This yield of 57% is considerable lower than all the other Fremy's salt oxidation steps in

which the compounds has a C-10 formyl group. It is interesting to note that during the course of this reaction, the reaction remained purple unlike most other Fremy's salt oxidations which after a short period of time, *i.e.* 1-2 hours turned orange in colour. This was also observed for compound (78) which was obtained in a modest yield of 66%. Such compounds (78) and (133) can be used as references for the biological testing as on reductive activation these compounds theoretically should have no antitumour activity (no alkylation sites at C-1 or C-10).

Secondly, quinone analogues were synthesised which had the C-10 leaving groups, urethane (138), and acetate (139). Unlike the above compounds (78) and (133), these molecules (138) and (139) on reductive activation might have some antitumour activity, as they possess one alkylation site, *i.e.* C-10 group. Hence, the next stage, was formylation of 3H-pyrrolo[1,2-a]indoles (130), and (131). Initially, formylation at the C-9 position of (130) and (131) was attempted under exactly the same conditions as previously discussed, using *N*-methylformanilide, phosphorus oxychloride in refluxing 1,2-dichloroethane for 1 hour. Compound (130) was formylated to give the desired product (134) in 59% yield as shown in *Scheme 22*. This was not the case with (131), which just gave an inseparable mess on purification . However, by altering the reaction conditions to DMF / phosphorus oxychloride the desired product (135) was obtained in good yield, 73%, as shown in *Scheme 22*.[85]

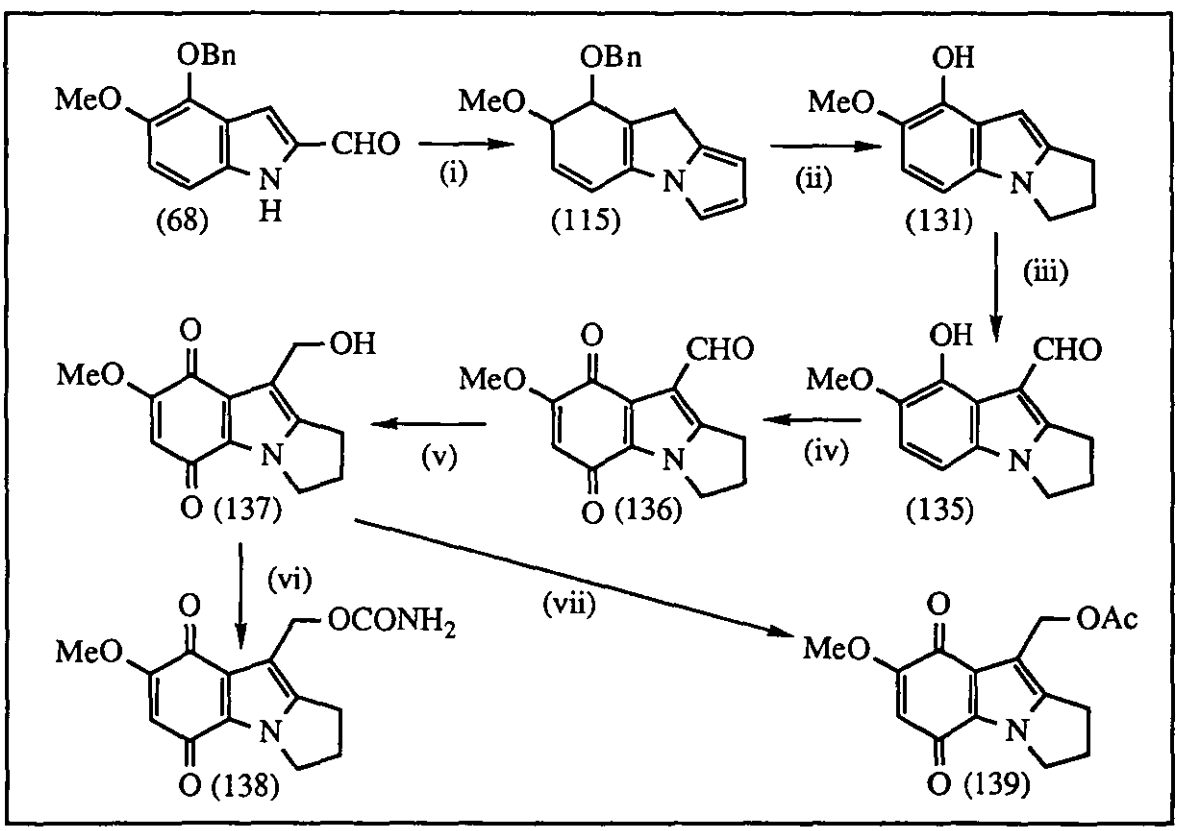
Scheme 22



The phenol (135) was subsequently oxidised with Fremy's salt in a mixture of water and

acetone which was buffered with sodium dihydrogen phosphate to give the tricyclic quinone (136), in excellent yield (89%). Reduction of the tricyclic system (136) was achieved by using sodium borohydride in methanol to afford the desired quinone alcohol (137) in 65% yield. Transformation of the quinone alcohol (137) into the desired product (138) was achieved over two steps; firstly forming the phenyl carbonate of the alcohol (137), using phenyl chloroformate in pyridine, followed by ammonolysis of this phenyl carbonate to give the urethane (138) directly, in a yield of 68% from (137), as shown in *Scheme 23*. The final urethane (138) was also a brick red solid which was totally insoluble in ethyl acetate, dichloromethane, and only sparingly soluble in DMF. It is quite remarkable how the solubility in organic solvents of the different compounds varies with and without the cyclopropane ring *i.e.* (58) of the MeO-Me_2 series being the most soluble and the present compound (138) being the least. It was also possible to synthesise the acetyl derivative (139) by treatment of the alcohol (137) with an excess acetic anhydride in pyridine, which was stirred overnight, in 78% yield, as shown in *Scheme 23*.

Scheme 23



Reagents: (i) NaH, THF; CH_2=$CHP^+(C_6H_5)_3Br^-$; (ii) H_2 / Pd-C; (iii) $POCl_3$, DMF; (iv) Fremy's salt; (v) $NaBH_4$, MeOH; (vi) $ClCO_2Ph$, pyridine; NH_3, CH_2Cl_2; (vii) Ac_2O, pyridine.

In Summary: In this Chapter a range of different cyclopropamitosenes have been synthesised, of which the key step of the synthetic route being a 1,3-dipolar addition of a diazo compound to an alkene. In the mitosene family, the key step was a modified Wittig reaction. All the final compounds are currently under biological investigation and electrochemical data has been recorded. From the onset of the work it was envisaged that the antitumour activity could be related to structural modification in the parent final urethane (57). Some of the final urethanes were subjected to exchange reactions at the C-7 position which shall be discussed in Chapter 3. The presence of the 7-methoxy group is important, from the observation that the quinone alcohol (59) was so reactive that it decomposed on attempts to generate the urethane (113), but the reason for this is still unclear.

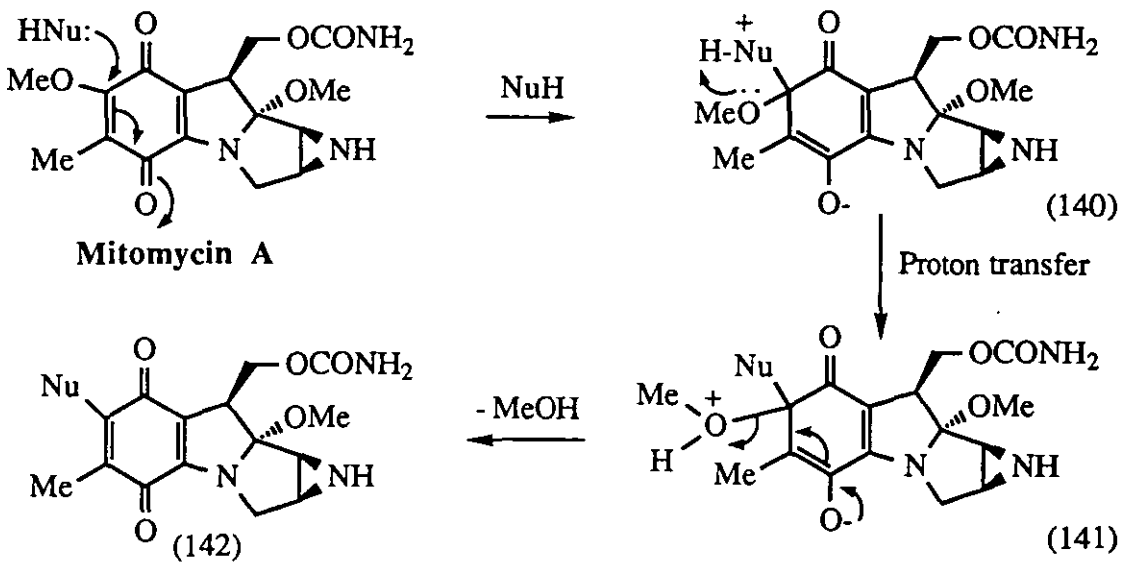
CHAPTER 3

C-7 EXCHANGE REACTIONS OF CYCLOPROPAMITOSENES AND MITOSENES

3.1. Introduction to C-7 Exchange Reactions

Functional group manipulation at the C-7 position of mitomycins has long been a concern of the medicinal chemistry community. Literature precedent by previous authors suggests that position 7 of MMC controls the reduction of the quinone ring, thus offering a chance to gain some selectivity between normal cells and certain cancer cells (hypoxic cells). Hence, in this Chapter, a variety of structural groups were explored as substituents at the 7 position of cyclopropamitosenes and mitosenes. The most important nonhydrolytic reactions of mitomycins, has been the replacement of the 7-methoxy group with other substituents, especially amines and alkoxides.[6, 23] The reaction with any nucleophile can be envisaged as a three step addition-elimination reaction. Initially a nucleophile adds at the C-7 end of the conjugated carbonyl system (Michael type reaction) affording (140), a proton is then transferred to the 7-methoxy group generating intermediate (141), and methanol is eliminated to afford the desired 7-substituted mitomycin A derivative as shown in *Scheme 24*.[6]

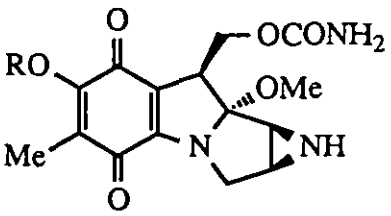
Scheme 24: Reaction of Mitomycin A with a Nucleophile



3.2. Synthesis of 7-Alkoxycyclopropamitosenes

The mitomycins, including their derivatives, can be classified into three groups; (i) mitosanes, (ii) aziridinomitosenes, and (iii) mitosenes. Among the mitosanes, mitomycin A has the greatest antibacterial activity against various bacteria and is the most cytotoxic. Therefore, it has been suggested that mitomycin analogues such as 7-alkoxymitosanes (143) might have interesting pharmacological properties. Extensive studies by two separate groups, Urakawa, Nakano, and Imai in Japan, and Iyengar, Remers, and

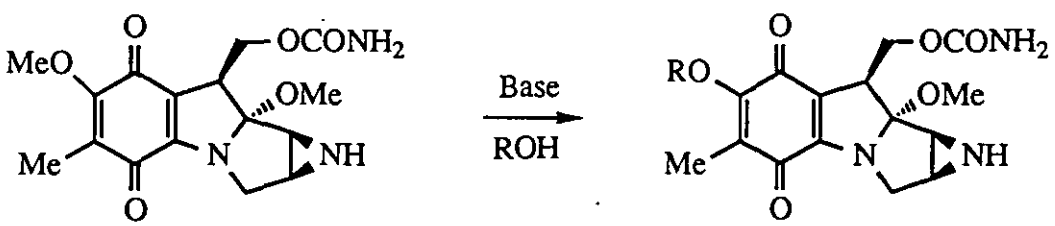
Bradner in the United States have led to a useful one-step synthesis of various 7-alkoxymitomycins (143).



7-Alkoxymitomycins (143)

Prior, to investigations by the above two groups, 7-alkoxymitomycins (143) were synthesised by treatment of a 7-hydroxymitosane with a diazoalkane in ether at -10°C for 5 hours. This procedure had not been investigated beyond the use of diazomethane to prepare mitomycin A from MMC.[5] Iyengar *et al.* were the first to investigate the scope of the diazoalkane reaction and the authors concluded that mitomycin A and 7-ethoxymitosane were isolated in satisfactory yields, but it was not a satisfactory method for larger diazoalkanes.[86]

In 1980 the Japanese group, developed a useful one-step synthesis of various 7-alkoxymitosanes (143) from naturally occurring mitomycins having 7-methoxy substituents.[87] In this procedure, 7-methoxymitosanes (mitomycin A and one reaction with mitomycin B) underwent alcoholysis in the presence of a small amount of base (potassium hydroxide) to afford the corresponding 7-alkoxymitosanes (143), as shown below. The mass effect of the solvent drives the reaction towards completion. Even so, the new alkoxide must not be a better leaving group than methoxide, for example 2-fluoroethanol fails to react with 7-methoxymitosanes.[6] The various alcohols used were all acyclic without any other functional groups present except one with an ether group ranging from 2 to 10 carbons in length.



Mitomycin A

7-Alkoxymitomycins (143)

In 1987, Iyengar *et al.* extended this idea by synthesising 7-alkoxymitosanes (143), derivatives of mitomycin A which contained various functional groups including halogen, olefin, acetylene, alcohol, ether, sulfide, disulfide, and tertiary amines. Primary and

secondary amines could not be used because they would give 7-amino (MMC) analogues.[87]

From the onset of our work, it was important to synthesise derivatives of the final cyclopropamitosenes (57) and (58) to investigate what effect structural modifications have on the antitumour activity. Following the procedure described by Iyengar *et al.* for mitomycins, we attemted alkoxide exchange reactions with the novel cyclopropamitosene (57).[86] In this method, cyclopropamitosene (57) was treated with 1.6% potassium hydroxide in the new alcohol as solvent to generate novel 7-alkoxycyclopropamitosenes (144), as shown below. The various alcohols used were; 2-methoxyethanol, ethylene glycol, ethanolamine, and *N*-acetylethanolamine, as shown in *Table 7*. Polyhydroxy compounds such as ethylene glycol and most of the other alcohols present problems because of their viscosity, especially in preparing the 1.6% potassium hydroxide solution in the alcohol. Usually it was necessary to sonicate the 1.6% potassium hydroxide solution for a considerable amount of time before the solution became clear. This procedure does not work with solid alcohols.

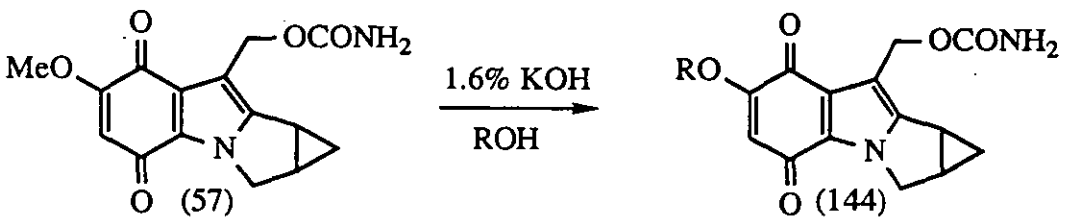
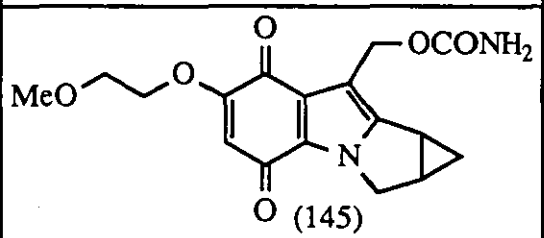


Table 7

Quinone	Alcohol	Product	Yield (%)
(57)	MeO–OH		70
(57)	HO–OH	No desired product	-
(57)	HO–NH_2	No desired product	-
(57)	HO–NHAc	No desired product	-

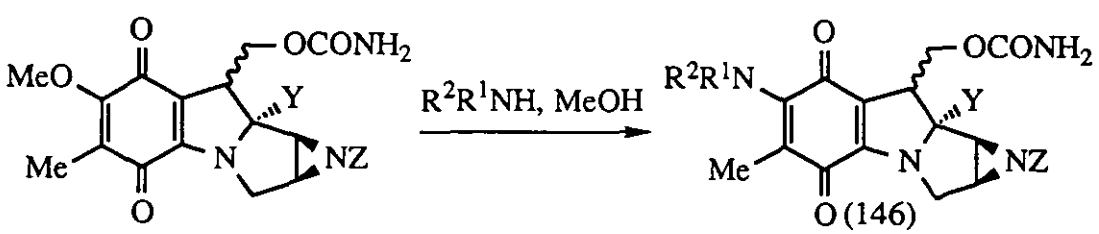
From *Table 7* it can be seen that of the various alcohols used, only 2-methoxyethanol

formed the desired exchanged 7-alkoxycyclopropamitosene (145) (70%), which exhibited the characteristic orange colour. It is not surprising that ethanolamine and *N*-acetylethanolamine did not afford the desired 7-alkoxycyclopropamitosene (144), and this is in accordance with previous results presented by Iyengar *et al.*.[86] The authors concluded that primary and secondary amines could not be used because they would give 7-amino (MMC) analogues. When treating the cyclopropamitosene (57) with either ethanolamine or *N*-acetylethanolamine the solution went purple in colour and a complex mixture was obtained which could not be purified by column chromatography on such a small scale (5-10 mg). Again, when cyclopropamitosene (57) was treated with ethylene glycol in base, a complex mixture was obtained. It is possible that the desired 7-alkoxycyclopropamitosene (144) was formed but then immediately dimerised.

3.3. Cyclopropamitosene and Mitosene Analogues with Substituted Amines at the C-7 Position

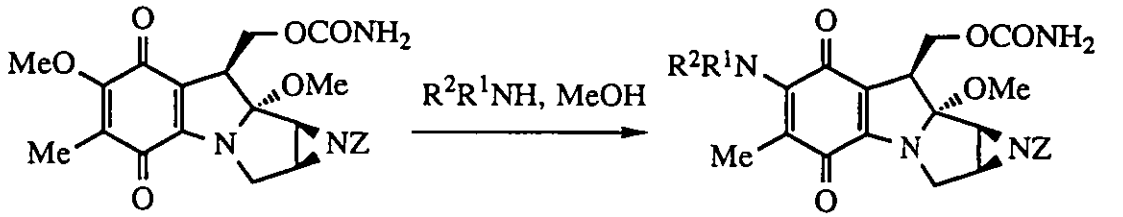
Literature precedent on 7-substituted mitosane and mitosene analogues of MMC suggests that antitumour activity could be modified and improved in some cases by varying the substituents on the quinone ring, especially with amines. The quinone reduction potentials of these aminoquinone derivatives of mitomycins and mitosenes show a wide range of values. As already discussed in Chapter 1, quinone reduction is essential for the bioreductive activation of mitomycins. As the 7-substituent has a controlling factor on the reduction of the quinone ring, this offers a chance to gain some selectivity between normal and certain cancer cells (hypoxic). From the onset of the work we envisaged that 7-(substituted-amino)cyclopropamitosenes and mitosenes could be synthesised and the antitumour activity both *in vitro* and *vivo* could be determined.

In 1971, Kinoshita *et al.* synthesised 7-aminomitomycins (146) of mitomycin A and mitomycin B by treatment of the mitomycins with an excess of primary and secondary amines in methanol, as shown below.[62]



Where: Y = OMe, Z = H, Mitomycin A, *trans*
Y = OH, Z = Me, Mitomycin B, *cis*

The bulk of the investigative work on this particular exchange reaction, *i.e.* the 7-methoxy group exchanged with amines, has mainly be undertaken by Remers, Bradner and Iyengar. In 1981, Iyengar *et al.* did an extensive study in which the authors synthesised a wide range of MMC and porofiromycin 7-(substituted-amino) derivatives.[88] Initially, MMC and porofiromycin were converted into MMA and *N*-methylmitomycin A (13). These were then subsequently treated with a variety of amines (5 equivalents) in methanol, including aziridines, allylamines, propargylamines, chloroalkylamines, hydroxyalklamines, aralkylamines, and heterocyclic amines, as shown below.

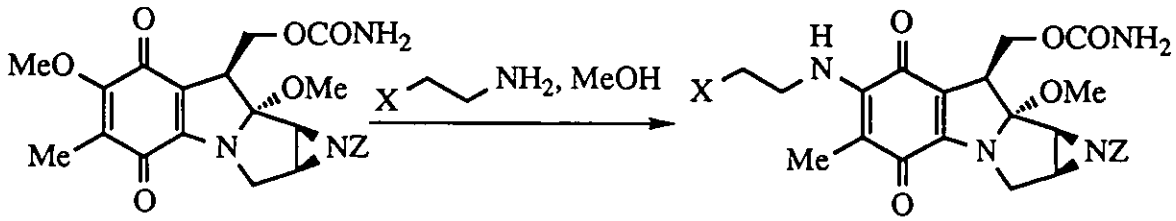


Where: Z = H, Mitomycin A
Z = Me, *N*-Methylmitomycin A (13)

MMC
Porofiromycin

7-(Substituted-amino)mitomycin (147)

Certain analogues were found to be superior to MMC in potency, efficacy, and therapeutic ratio. The most active substituents at the mitosane 7 position included aziridine, 2-methylaziridine, furfurylamine, and 3-aminopyridine.



Where: Z = H, Mitomycin A
Z = Me, *N*-methylmitomycin A (13)

MMC
Porofiromycin

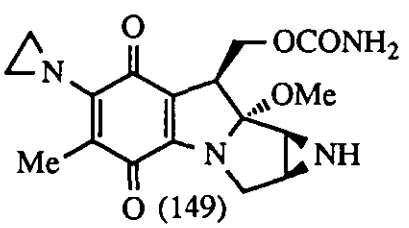
7-[(2-Substituted-ethyl)-amino] mitomycin (148)

Where: X = F, Cl, Ph, *p*-HO-Ph MeO, C_2H_5S, HS, NC, NH_2, C_2H_5.

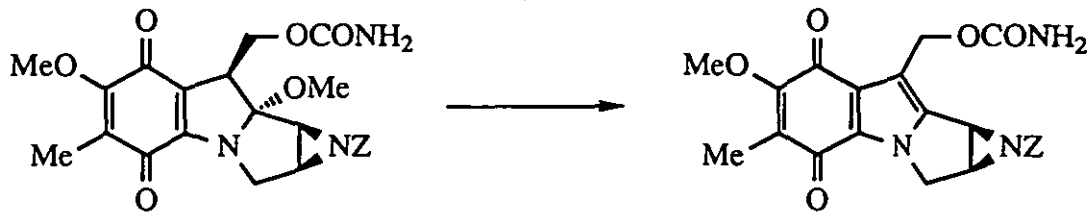
In 1983, Iyengar *et al.* synthesised a series of 7-[(2-substituted-ethyl)-amino]mitosanes (148) of MMC and porofiromycin which were screened in standard antitumour

systems.[89] The authors rationalised that one of the structural types which showed a good potential for analogue development was substituted ethylamine at position 7. Their approach to analogue development was to prepare an extended series of compounds in which the 2-position of the ethylamino group was substituted with a variety of different functional groups, as illustrated above. These groups were chosen to give compounds with a wide range of lipophilicity. They prepared these compounds by the same method as above, which involved treatment of a methanol solution of mitomycin A or its *N*-methyl homologue with excess amine.[88] The authors concluded that a number of the 7-[(2-substituted-ethyl)amino]mitosanes (148) that had been synthesised were found to be active against P-388 and other mouse tumours.

Prior to 1983, most of the substituted 7-aminomitosanes had been synthesised from primary amines. Consequently, Iyengar *et al.* undertook the preparation and testing of an extended series of secondary amines.[90] This series includes acyclic compounds and cyclic compounds with three, four, five, and six-membered rings. The desired secondary amino analogues were prepared by treatment of mitomycin A in anhydrous methanol or dichloromethane with the appropriate amine or amine hydrochloride. In a few cases, especially with large amines, the reaction was slow, and a trace of solid potassium carbonate was added to accelerate it. Pyrrole and indole failed to react with mitomycin A. This is not surprising in view of their poor nucleophilicity. The authors were unsuccessful in synthesising secondary amines containing four-membered rings. The 7-aziridino analogue of MMC (149) as shown below is known for its high potency against experimental tumours. Decreases in both the potency and antitumour effectiveness resulted when a methyl group was added to the 7-aziridine. The exact reason for this effect is still unclear, but it could be due to the size, lipophilicity, or electron-donation property of the methyl group. This biological result will be interesting to compare with our 7-substituted aziridine and 2-methylaziridine cyclopropamitosenes and mitosenes, which will be discussed in Chapter 5.



(149)



Where: Z = H, Mitomycin A
Z = Me, *N*-Methylmitomycin A (13)

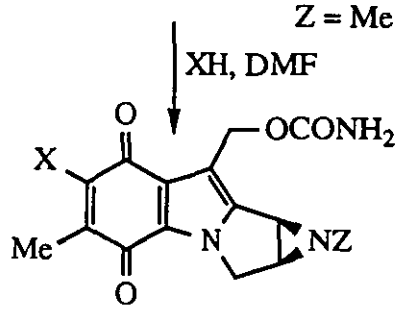
7-Methoxy-aziridinomitosene, Z = H (150)
Z = Me (17)

Where: X =  , Z = H / Me

X =  , Z = H / Me

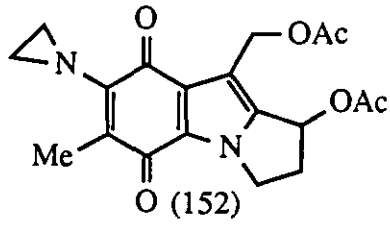
X = H_2N, Z = H / Me

X = $HC{\equiv}CCH_2NH$, Z = H



Z = H, (151 a)
Z = Me, (151 b)

In a recent paper, Iyengar *et al.* extended their studies to aziridinomitosenes (150) and (17), as shown above.[91] 7-Methoxy-1,2-aziridinomitosenes (150) and (17) were prepared from mitomycin A and its *N*-methyl homologue (13) by catalytic reduction followed by air oxidation. Treatment of these products with amines (an excess, 3 equivalents) in DMF, which was monitored by TLC under nitrogen, including ammonia, aziridine, 2-methylaziridine, and propargylamine gave the corresponding 7-(substituted-amino) derivatives (151a) and (151 b), as shown above. Screening of the compounds against P-388 leukemia cells in mice revealed some good activity. Orelemans *et al.* recently synthesised a 7-(aziridin-1-yl)mitosene (152), as shown below.[66]



(152)

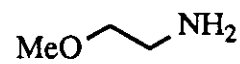
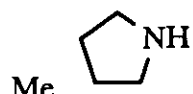
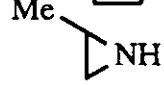
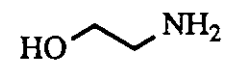
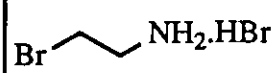
It can be seen from the literature, that 7-(substituted-amino) mitosanes, aziridinomitosenes, and mitosenes were synthesised from primary and secondary amines. The 7-aziridino analogues of these compounds in particular showed high potency against experimental tumours. From the onset of our project, some of the objectives were to synthesise analogues of MMC in an attempt to (i) enhance its antitumour activity but decrease the side effects and / or (ii) analogues that are more specific for hypoxic cells and not normal cells, preferentially at low drug concentration To fulfil these objectives

we decided to synthesise novel 7-(substituted-amino) cyclopropamitosenes and mitosenes. This was achieved by following the methodology established by Iyengar *et al.*.[91] Initially the cyclopropamitosenes (57), (58), and (78) will be discussed. The cyclopropamitosenes (57), (58), and (78) were treated with an excess of a variety of primary and secondary amines, ranging from acyclic amines like 2-methoxyethylamine, ammonia, ethanolamine, 2-bromoethylamine hydrobromide, to cyclic amines such as aziridine, 2-methylaziridine, and pyrrolidine, as shown below in *Equation 1* and *Tables 8*.

Equation 1

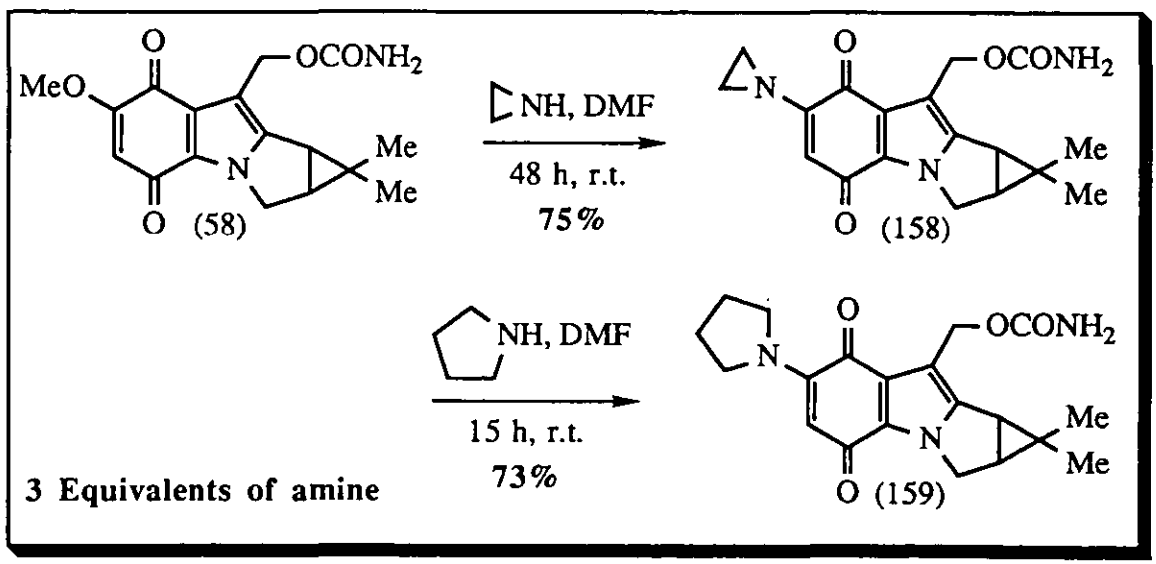
Normally 50-100 equivalents of amine

Table 8

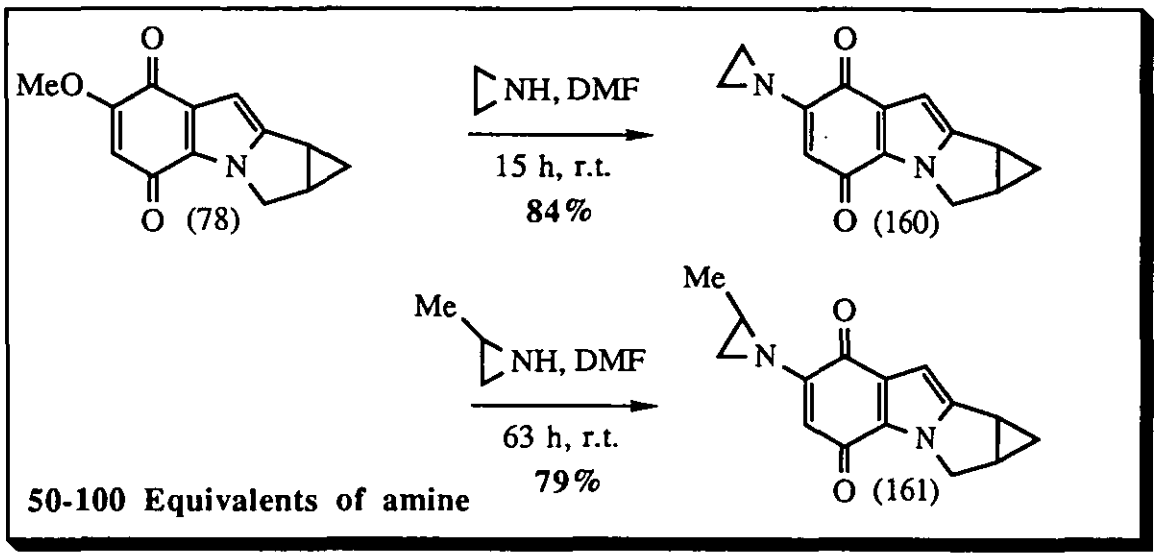
Quinone	Amine	Reaction Time (h)	Product Number	Yield (%)
(57)		15	(154)	64
(57)		15	(155)	73
(57)		48	(156)	68
(57)		15	(157)	84
(57)	0.88 NH_3	120	None	-
(57)	NH_3 (l)	-78°C (6 h) further 48 h @ r.t.	None	-
(57)		72	None	-
(57)		72	None	-

From *Table 8* it can be seen that the desired 7-(substituted-amino) derivatives of cyclopropamitosene (57) were formed when the amines: 2-methoxyethylamine, pyrrolidine, aziridine, and 2-methylaziridine were used, in yields varied from 64-84%.

The yields are a little variable since small scales are rarely indicative of the true yield. These 7-methoxy exchange reactions with amines were monitored by TLC. The reaction times varied from 15 hours to 120 hours depending on (i) the amine and (ii) the cyclopropamitosene used. All the amines and amine hydrobromides were commercially available except aziridine.[92] Initially, we attempted to synthesise the 7-(aziridin-1-yl) analogue (157) *in situ*. Unfortunately, this idea proved to be fruitless. An excess of sodium hydride was added to a stirred solution of 2-bromoethylamine hydrobromide in DMF at 0°C under nitrogen. After 2 hours the cyclopropamitosene (57) was slowly added dropwise to the stirred solution at 0°C, which was then stirred at 0°C for 2 hours. After this time the reaction mixture was stirred at room temperature for a further 72 hours. From ^{1}H NMR (250 MHz) no desired product (157) was observed. The only alternative was to synthesise aziridine according to a literature procedure,[92] from 2-aminoethanesulphonic acid (taurine) and 40% sodium hydroxide. Care was taken at all times when handling aziridine and 2-methylaziridine, as these compounds are extremely toxic, and potent alkylating agents of DNA. Attempts to prepare cyclopropamitosene analogues having an amino (NH_2) group at the C-7 position met with frustration. When the parent cyclopropamitosene (57) was treated with either (i) liquid ammonia at -78°C or (ii) 0.88 ammonia for a considerable amounts of time, the desired product was not isolated.[88, 91] Similarly with ethanolamine, which did not afford the desired product.[89] These results are not in accordance with the previous results presented by Iyengar *et al.* on MMC and porofiromycin analogues.[88, 89] In their studies the authors synthesised the desired 7-(substituted-amino) derivatives of MMC and porofiromycin, as previously stated. The disagreement in our results may be due to the different reactivity of the cyclopropamitosene (57) in contrast to the mitosanes. It was also possible to exchange the 7-methoxy group of cyclopropamitosenes (58) and (78) with amines, as shown below, in yields ranging from 73-84%.

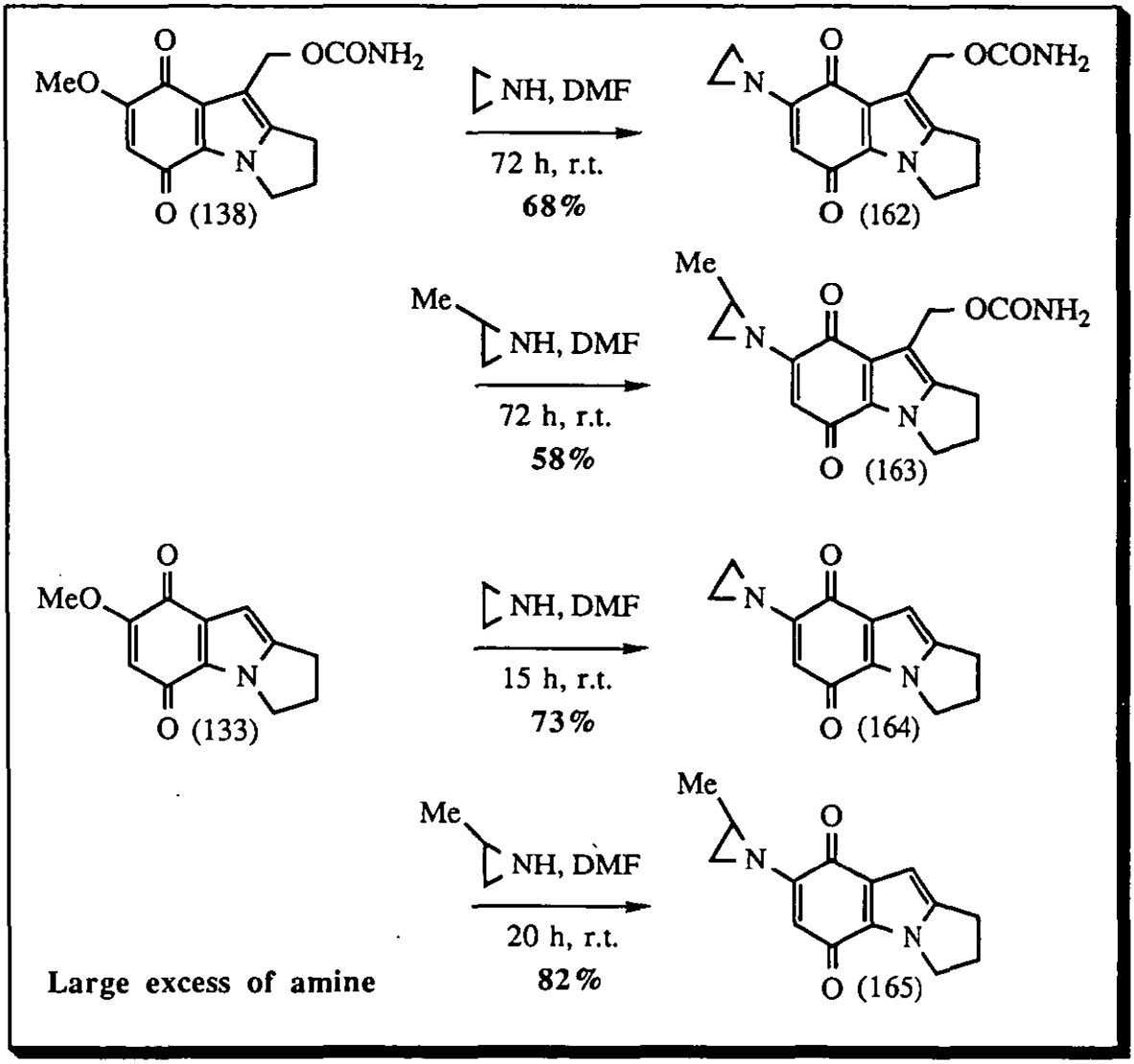


The 7-(2-methylaziridin-1-yl) analogue (161) was the only 7-(substituted-amino) derivative, which after purification by column chromatography and attempted recrystallisation from dichloromethane did not give a solid. This was peculiar, and compound (161) was afforded as a red oil, as shown below.



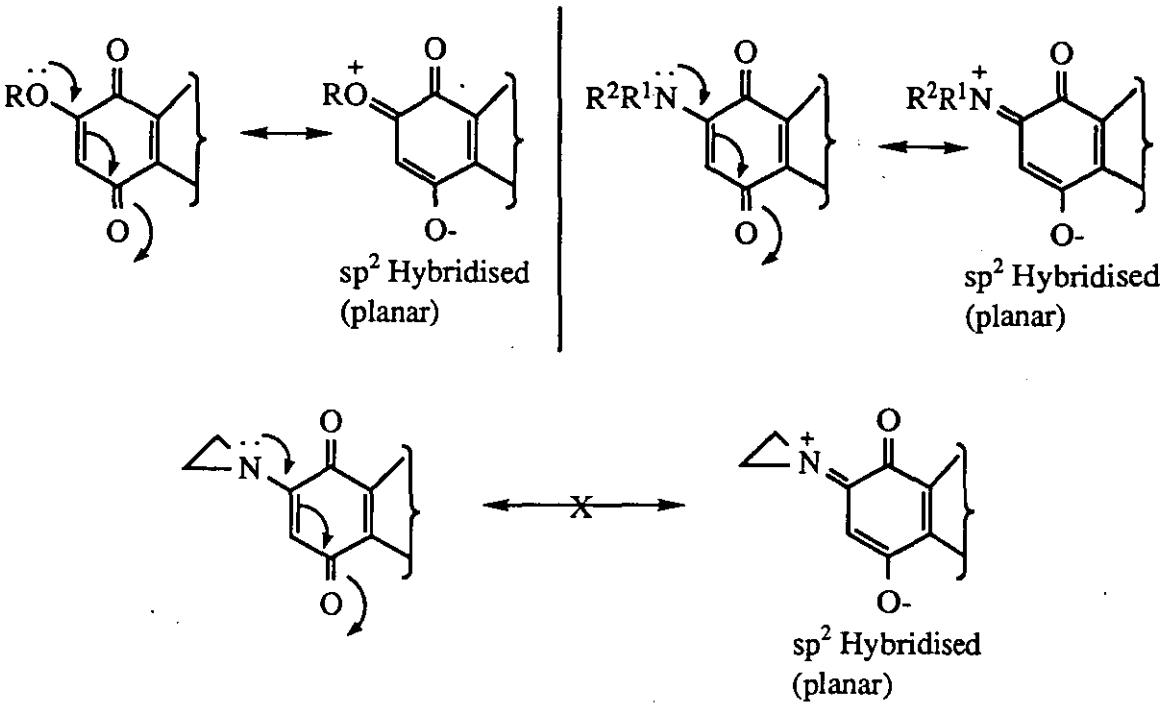
As previously stated cyclopropamitosenes (57) and (58) only have one alkylation site *i.e.* the C-10, however when the 7-methoxy group is exchanged for aziridine or 2-methylaziridine there are potentially two alkylation sites. The second site comes from opening of the aziridine ring. Cyclopropamitosene (78) now has one potential alkylation site. The correlation between antitumour activity of these cyclopropamitosenes and structural modifications at the C-7 position will be discussed in Chapter 5.

The 7-methoxy group of the novel mitosenes (138) and (133) were both exchanged with aziridine and 2-methylaziridine, to form the corresponding 7-(substituted-amino)mitosenes in yields varying from 58-82%, as shown below. All reactions were monitored by TLC. The structure of 7-(aziridin-1-yl)mitosene (162) was assigned by ^{1}H NMR and IR but unfortunately no satisfactory mass spectrum was obtained. Again, for both compounds there is the potential to increase the number of alkylation sites by one, which possibly could enhance the antitumour activity of the drugs. These points will be discussed in Chapter 5.



One of the peculiar properties of these novel cyclopropamitosenes and mitosenes is their colour. The colours, orange for the 7-alkoxy derivatives and purple for the the 7-(substituted-amino) analogues, except the aziridino derivatives which are red, are characteristic and useful in preliminary classification. These observations can be rationalised as follows. Firstly, the difference in colour between the 7-alkoxycylopropamitosenes and 7-(substituted-amino) derivatives, except the aziridino analogues will be considered. Both the oxygen of the 7-alkoxycyclopropamitosenes and the nitrogen of the 7-(substituted-amino) derivatives can rehybridise from sp^3 to sp^2, planar geometry, as shown below. However, since the lone pair of electrons on the nitrogen are not so tightly bound, this leads to more extensive delocalisation of the chromophore, and a shift to a longer wavelength. Hence the 7-(substituted-amino) analogues appear purple in colour. However, this is not the case with the aziridino derivatives. It is not favourable for the aziridine nitrogen atom to rehybridise from sp^3 to

sp^2, planar geometry, as this would increase the strain in the three-membered ring. Hence, in the aziridino analogues, there is no extensive delocalisation of the chromophore, and a shift to a shorter wavelength compared with other amino derivatives is observed. These compounds appear red in colour.



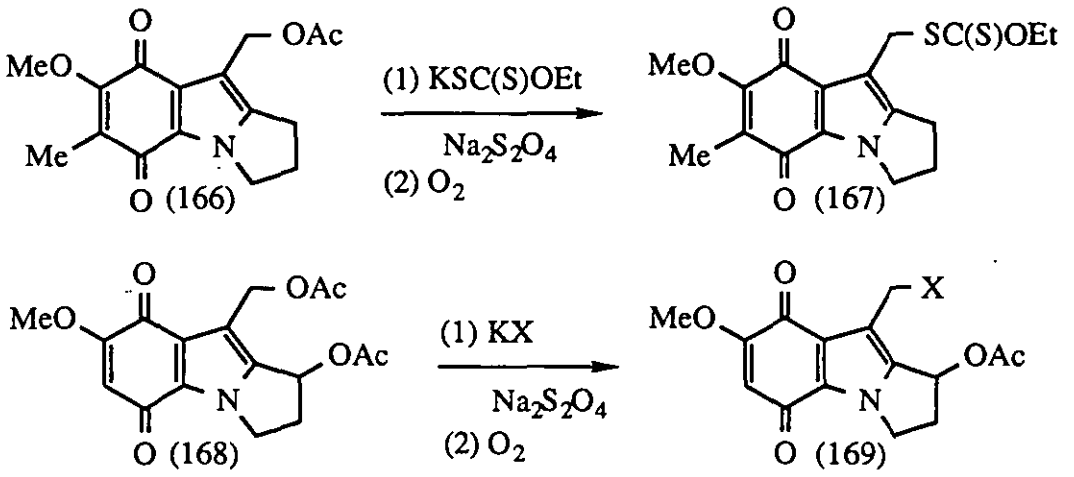
In Summary: The previous methodology for the synthesis of 7-alkoxymitosanes and 7-(substituted-amino)mitosanes and mitosenes was extended to the novel cyclopropamitosenes and mitosenes. This is the first time that 7-alkoxycyclopropamitosenes and 7-(substituted-amino) derivatives have been synthesised, in modest yields. When 7-(aziridin-1-yl) and 7-(2-methylaziridin-1-yl) analogues of either cyclopropamitosenes or mitosenes were synthesised, one extra alkylation site is gained by opening of the aziridine ring. The correlation between antitumour activity and structural modification at the C-7 position will be discussed in Chapter 5.

CHAPTER 4

ALKYLATION OF NUCLEOPHILES BY C-10 OF CYCLOPROPAMITOSENES AND MITOSENES

4.1. Bioreductive Alkylation as the Mode of Action of the Cyclopropamitosenes and Mitosenes.

From the outset of this work, one of the objectives was to undertake a study on cyclopropamitosenes, studying any possible antitumour activity relative to aziridinomitosenes, and hence enhance our understanding of the mechanism of action of MMC. In this Chapter the role of C-10 in cyclopropamitosenes and mitosenes as an alkylating centre under reductive conditions, will be discussed. Initially, we shall consider the proposed mode of action of MMC, as shown on next page in *Scheme 7*.[67] This proposed mechanism has previously been discussed in Chapter 1 and nothing else will be said about it here.

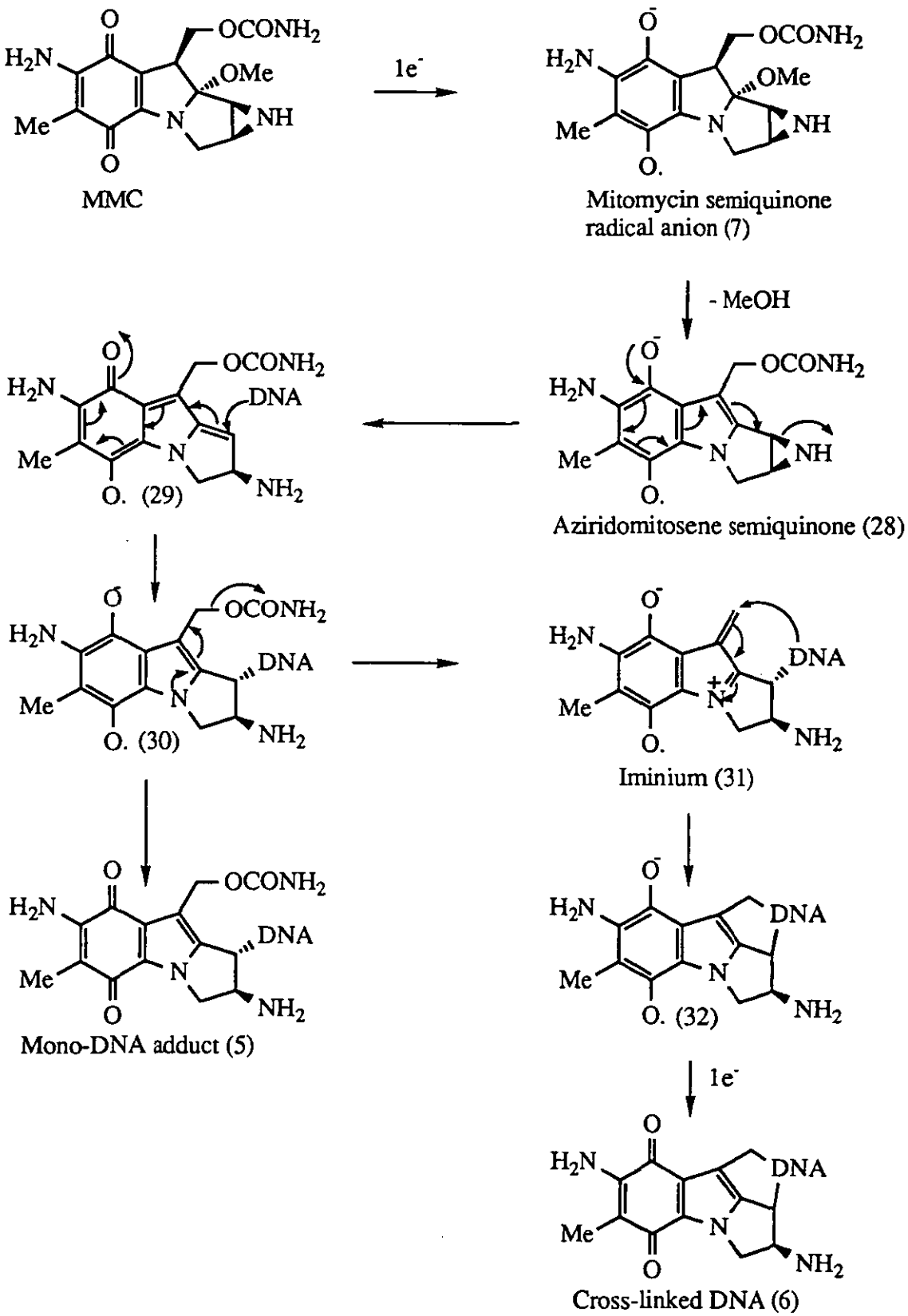


Where: X = SC(O)Ph (76%)
SC(S)OEt (73%)

A vast amount of mechanistic studies have been done using MMC. However, more recently literature has been published on the mechanism of alkylation at the C-10 position of mitosenes, two of which will now be discussed. Firstly, Orlemans *et al.*[66] have studied several mitosenes (166) and (168) for their reactions with nucleophiles under reductive conditions (sodium dithionite) to form the corresponding C-10 alkylated products (167) (92%) and (169), as shown above. The results of the experiments show that the biological activity of mitosenes is based on the mechanism of reductive activation. The authors concluded, from competition experiments, that when both the leaving groups at C-1 and C-10 were the same as in the mitosene (168), the nucleophile preferably adds to C-10 under reductive conditions with either potassium ethyl xanthate, or potassium thiobenzoate (a poorer nucleophile). The predominant substitution at the C-10 position is in sharp contrast with the difference in reactivity between the C-1 and C-10 position in MMC. In MMC C-1 is the preferred position of alkylation under reductive conditions

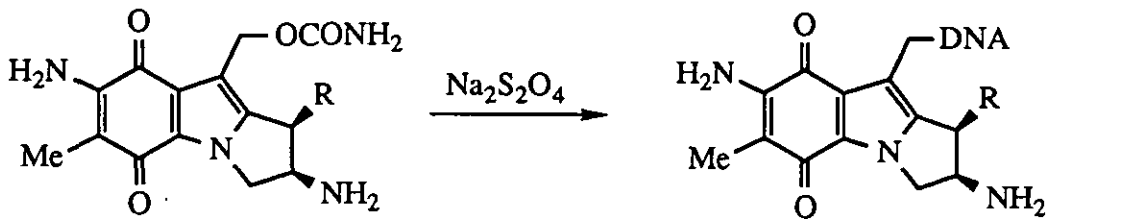
due to the more reactive aziridine substituent, as illustrated in Chapter 1.

Scheme 7: The proposed mechanism for the mode of action of MMC



Secondly, Iyengar, Dorr, Shipp, and Remers have shown recently that monoalkylation by C-10 (carbamate group) can occur for mitosene analogues that have no reactive C-1 functionality.[41] Sodium dithionite reduction of 2,7-diaminomitosene (8) or *cis*-2,7-diamino-1-hydroxymitosene (9) in the presence of calf thymus DNA resulted in alkylation of DNA to the extent of one molecule per 14 and 11 bases, respectively as shown in *Scheme 6*. However no covalent binding was observed on catalytic reduction. Reduction of each of these mitosenes (8) and (9) by sodium dithionite in the presence of 2'-deoxyguanosine gave monoalkylation on the 2-amino group of the nucleotide. These results are again consistent with a mode of action in which bioreductive activation of C-10 is followed by monoalkylation of DNA. The authors demonstrate that covalent binding between C-1 of mitosenes and DNA is not required to activate the molecule for C-10 alkylation.

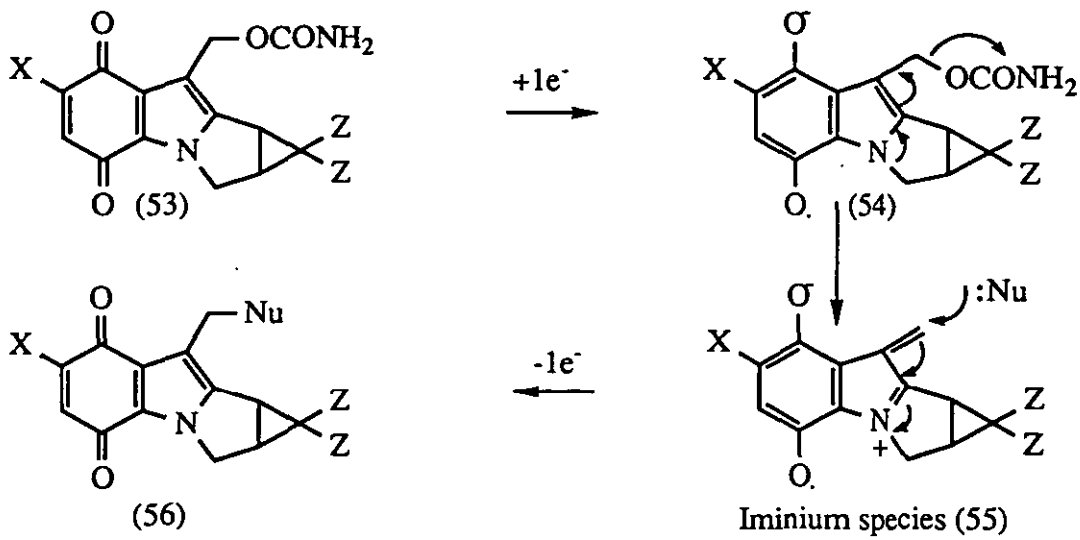
Scheme 6



Where: R = H (8)
R = OH (9)

Where: R = H (26) [1 molecule per 14 bases]
R = OH (27) [1 molecule per 11 bases]

From the outset of this project one of the objectives was to investigate the mechanism of alkylation at the C-10 position of cyclopropamitosenes and mitosenes. The proposed mechanism of alkylation under reductive activation conditions of these novel cyclopropamitosenes, in the presence of nucleophiles, can be envisaged as a process closely related to the second stage of alkylation by MMC.[67] The initial step is a single electron reduction of the cyclopropamitosene (53) to a semiquinone radical anion (54). The activated mitosene semiquinone formed (54) is now rendered electrophilic at C-10 and elimination of the carbamate group is assisted by the "push" from the indole nitrogen, generating an iminium species (55). The iminium species (55) in the presence of a nucleophile, followed by oxidative work-up, should yield the corresponding C-10 adduct (56), as illustrated below. Before reductive activation the lone pair of electrons on the indole nitrogen can be delocalised into the quinone ring, and hence the carbamate group at the C-10 position cannot be eliminated.

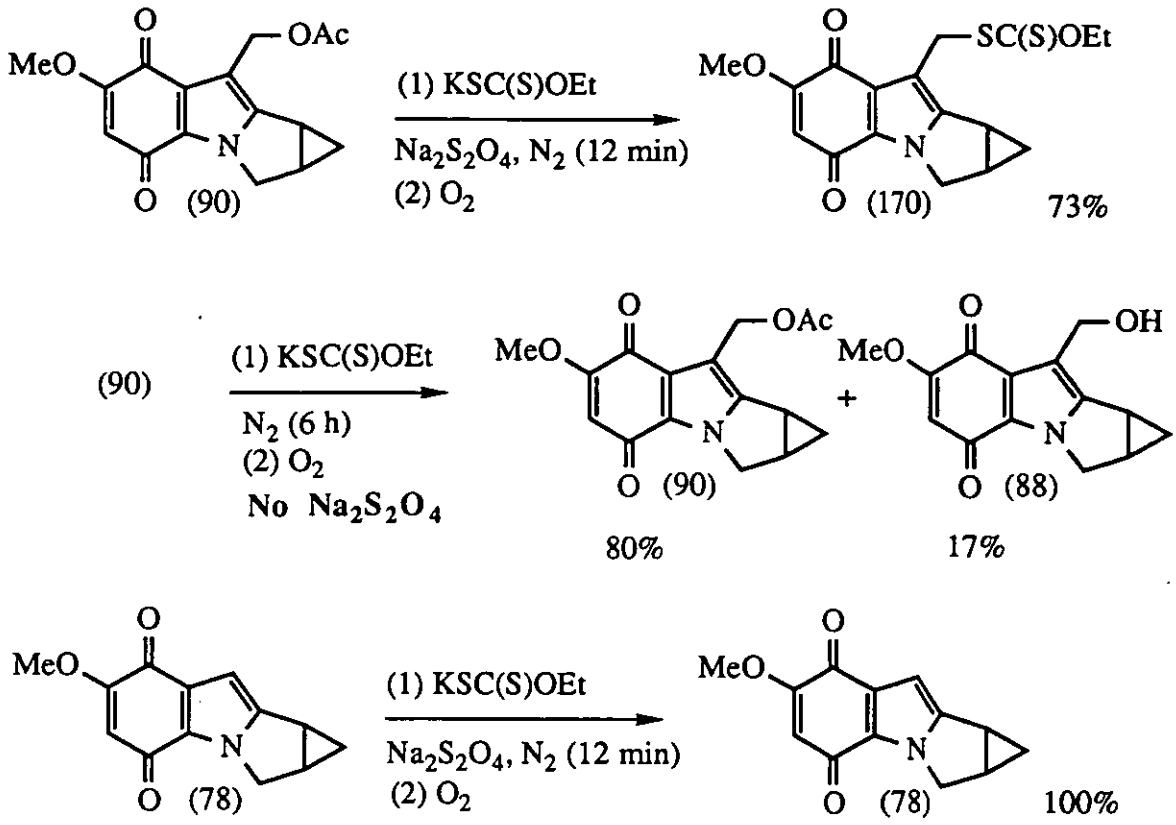


Where: (1) X = OMe, Z = H (57)
(2) X = OMe, Z = Me (58)

4.2. Reactions of Cyclopropamitosenes and Mitosenes with Nucleophiles under Reducting Conditions

To confirm that the C-10 alkylation reaction occurs by a bioreductive activation mechanism, similar to the the second stage of alkylation by MMC we designed a number of experiments. This was achieved by following the methodology established by Orelmans *et al.* for mitosenes.[66] A variety of nucleophiles were reacted with cyclopropamitosenes and mitosene (138) under reductive conditions. In initial studies, potassium ethyl xanthate was used as the nucleophile. In these cases the reactions were conducted in a mixture of dichloromethane / methanol / water (1:1:1) with sodium dithionite as a reductant under nitrogen for a short period of time (varying from 10 min-12 min). Initially, cyclopropamitosene (90) was used and after reoxidation by air, the product (170) was purified by column chromatography, to give the corresponding ethyl xanthate substituent at C-10 (170), which was isolated in a yield of 73%, as shown below, in *Scheme 25*, reaction 1.

Scheme 25

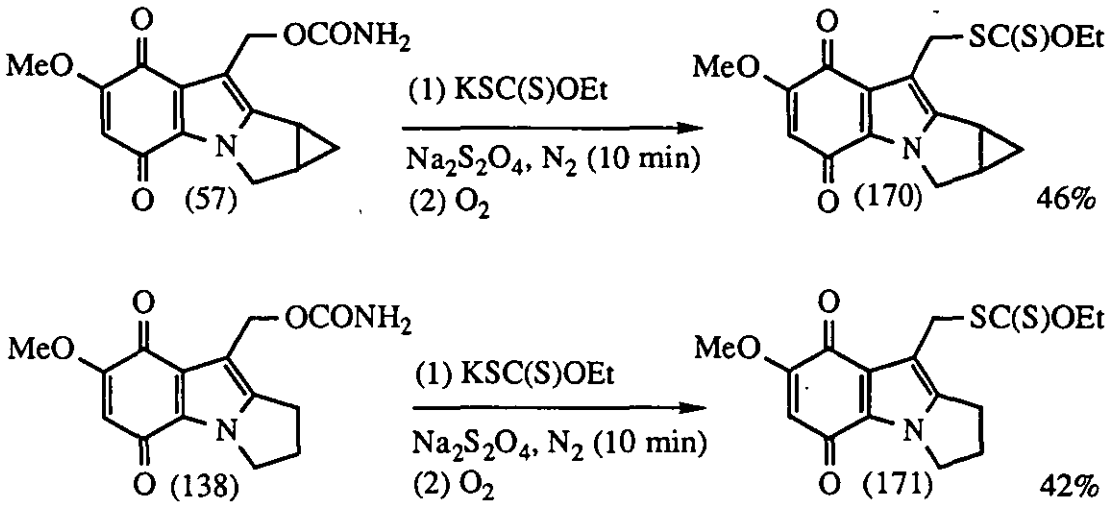


It should be emphasised that in all these cases the presence of sodium dithionite is a prerequisite; without this reductant no reaction takes place. This point is illustrated in reaction 2, *Scheme 25*. In this experiment the reaction conditions were the same as the previous experiment except that **no sodium dithionite** was added and the reaction time was lengthened from 12 minutes to 6 hours. The product profile was completely different, 80% starting material (90) was recovered and 17% of the quinone alcohol (88), which was generated by hydrolysis of the corresponding ester (90). This is in total contrast to the previous experiment , in which 73% of the desired C-10 alkylated product (170) was isolated. Hence, these results suggest that the reductive activation of cyclopropamitosene (90) proceeds *via* an iminium ion intermediate, as already postulated, and not just nucleophilic displacement of the acetyl group by the incoming nucleophile *i.e.* potassium ethyl xanthate by an S_N2 mechanism. In our studies we also investigated whether or not the cyclopropane ring opened in a similar manner to the aziridine ring in MMC. In experiment 3 of *Scheme 25*, cyclopropamitosene (78) was reacted with ethyl potassium xanthate under reductive conditions, similar to that of reaction 1 in *Scheme 25*. As this compound (78) has no alkylation sites *i.e.* no C-10, only starting material (78) should be generated unless the cyclopropane ring opens. However, after oxidative work-up the only product isolated was indeed the starting

material (78). This result indicates that during reductive activation the cyclopropane ring remains intact.

The next logical extension would be to study reactions of potassium ethyl xanthate with cyclopropamitosene (57) and mitosene (138) which possess the C-10 carbamate group similar to MMC, under reductive conditions. The experiments were conducted under exactly the same conditions as reaction 1 of *Scheme 25*. After oxidative work-up, the only product obtained in both cases was the ethyl xanthate substituent at C-10 of cyclopropamitosene (57) and mitosene (138) *i.e.* (170) 46% and (171) 42% yields respectively, as shown below in *Scheme 26*. The yields with cyclopropamitosene (57) and mitosene (138) are considerably lower than that of cyclopropamitosene (90) with the C-10 acetate group. The reason for this is still unclear, but no other products were isolated on work-up.

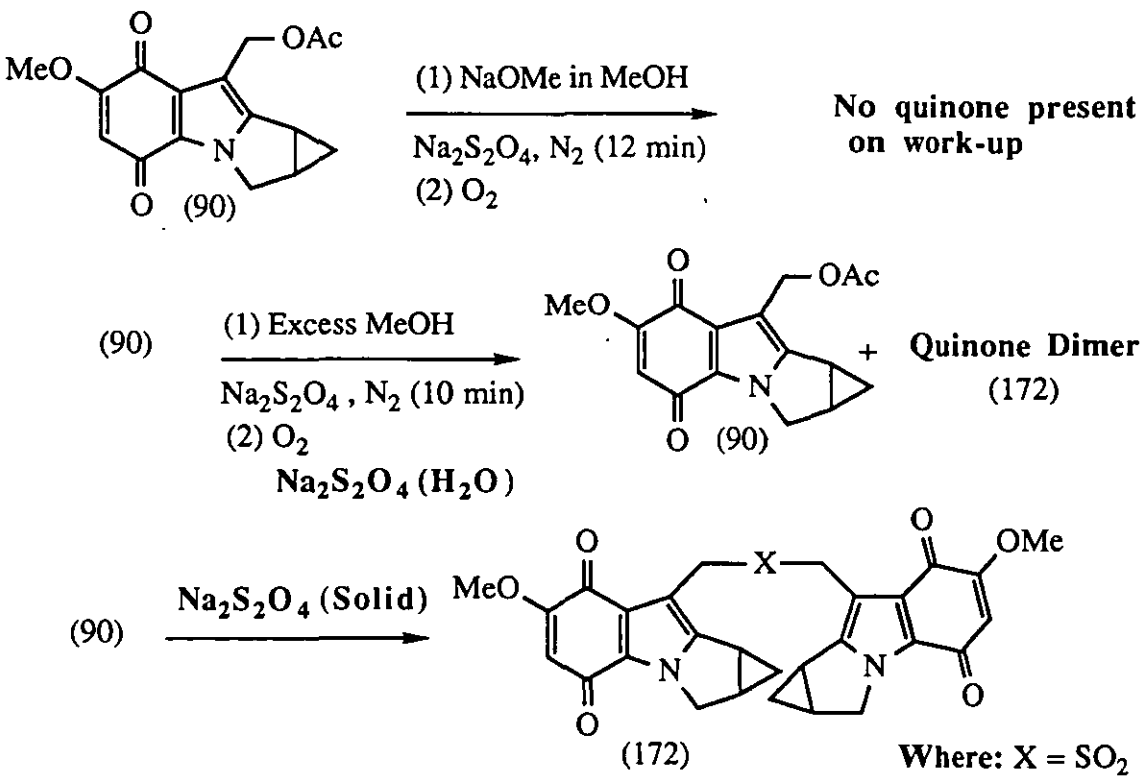
Scheme 26



It was then decided to see how useful this procedure is for bonding cyclopropamitosenes at the C-10 position to other reagents of varying nucleophilic strength *i.e.*, alcohols, amines, 2'-deoxyguanosine, and ultimately DNA. However, all attempts to prepare the C-10 monoalkylated product (173) with either sodium methoxide or methanol under reductive conditions met with frustration. Initially, the reactions were conducted under exactly the same conditions as reaction 1, *Scheme 25*, except that sodium methoxide or methanol was used as the nucleophile instead of potassium ethyl xanthate, as illustrated for reaction 1 and 2, in *Scheme 27*. After oxidative work-up, baseline material was formed when methoxide was used as a nucleophile, and the cyclopropamitosene (90) was recovered with an a trace amount of a dimer (172) with excess methanol. Accidentally, when solid sodium dithionite was added to the stirred solution of cyclopropamitosene

(90) in the presence of excess methanol, only one product was obtained, a dimer (172) (28%), as shown below in *Scheme 27*. The structure of compound (172) has been tentatively assigned by comparing all the spectroscopic data. Firstly, from the ^{1}H (400 MHz) spectrum the entire basic cyclopropamitosene skeleton is intact with a C-10 methylene group. The two protons of the C-10 methylene group have different chemical shift values of 4.77 and 4.52 ppm respectively. Secondly, from the ^{13}C NMR (100.6 MHz) spectrum there are more carbon peaks relative to the protons present (most of the carbon peaks are doubled up), thus indicating two diastereomers of the dimer being present. Mass spectral data also supports the dimer structure. Compound (172) had a molecular weight of 548, and we are proposing that X $=SO_2$, to correspond with the observed mass ion of 549 ($\underline{MH}^+$).

Scheme 27



As this reaction failed to give the desired C-10 monoalkylated (173) product using sodium dithionite as the reductant, we decided to try an alternative reducting agent. Again, all attempts at this reaction proved to be fruitless, as shown below in *Table 9*. The hydrogenation reactions will now be discussed. Reductive activation of the final urethane (57) with either palladium on activated carbon [34,41] or platinum oxide [27,93,94] in the present of methanol failed to give the desired C-10 adduct (173), but a complex mixture. The desired product (173) as shown below was obtained, as evidenced by

TLC, but unfortunately there was not enough sample to be isolated and characterised. Alternatively, by varying the reaction times, and conducting the reactions at 3 atmospheres pressure, a complex mixture was still obtained. These various reactions conditions are summarised below, in *Table 9*.

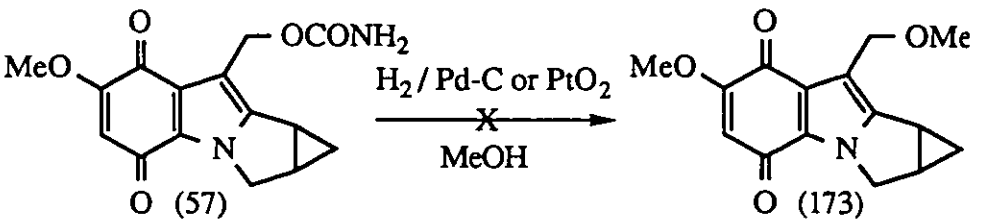
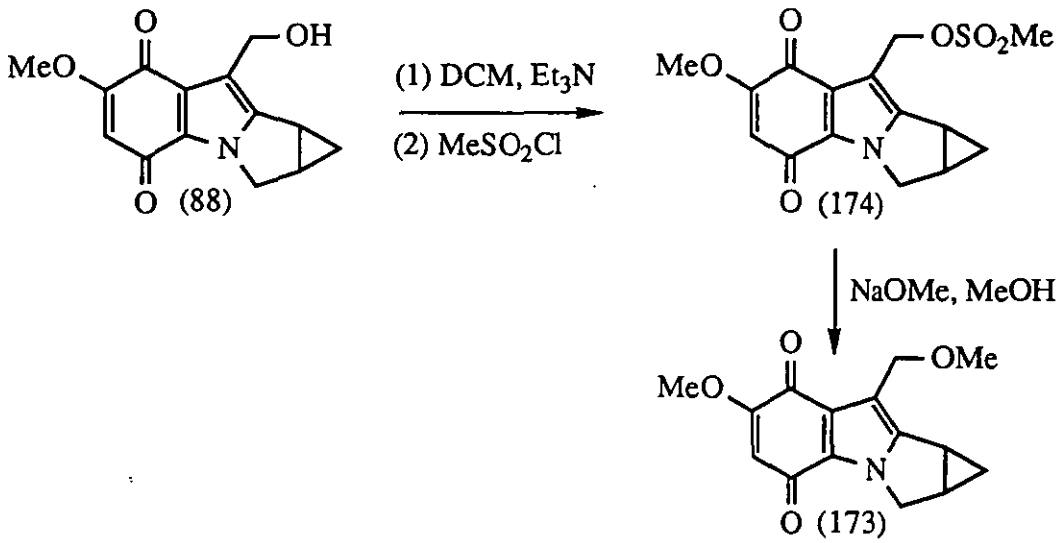


Table 9

Quinone	Reagent	Reaction Conditions	Product
(57)	H_2 / Pd-C	40 min at atmospheric pressure	6 Spots by TLC
(57)	H_2 / Pd-C	Monitored the reactions by TLC Took out samples at the following time intervals - 5, 10, 20, 60, 90, and 120 min	6 Spots by TLC
(57)	H_2 / PtO_2	7 and 30 min at atmospheric pressure	Complex mixture
(57)	H_2 / PtO_2	30 min at 3 atm. pressure	Complex mixture

Compound (173) was prepared by an alternative route, but with extreme difficulty. Initial, attempts to prepare this compound (173) by standard methods from the quinone alcohol (88), were unsuccessful in our hands. Oxygen methylation was attempted using sodium hydride in DMF or *N, N*-disopropylethylamine in dichloromethane, and subsequent quenching with methyl iodide. Unfortunately, in both cases, the desired product (173) was not present, only baseline, and unreacted starting material were obtained respectively. By altering the base and solvent to potassium carbonate in refluxing acetone, again unreacted starting material was recovered. Transformation of the alcohol (88) into the required product (173) was achieved over two steps; the resultant

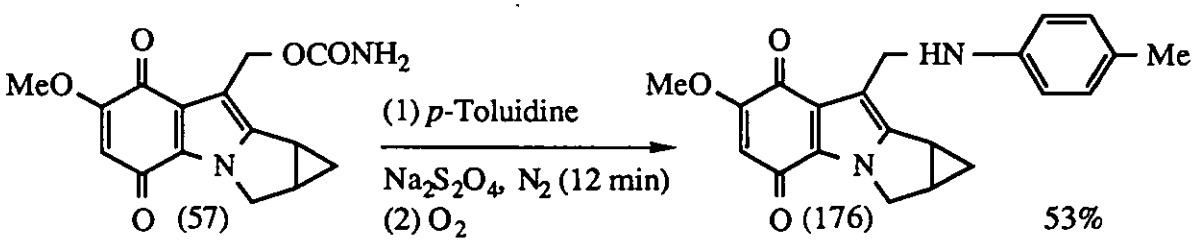
alcohol (88) was methanesulphonated to give (174) in good yield (69%) by treatment of the quinone alcohol (88) in dichloromethane with triethylamine and then quenching with methanesulphonyl chloride.[95] This compound (174) was not stable, and during chromatography on silica it regenerated the starting alcohol (88). Hence the methanesulphonate (174) was used without purification. The reaction of the sulphonate (174) with base, sodium methoxide in methanol resulted in the successful nucleophilic substitution to give the desired product (173) in 71% yield, as illustrated below.



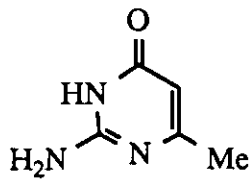
It appears from all the above experiments that both methoxide and methanol are poor nucleophiles with cyclopropamitosenes (90) and (57) under reductive conditions using a variety of reductants. It was possible to synthesise the desired compound (173) but by an alternative route. From comparing the TLC of some of the reductive activation reactions with an authentic sample of (173), it was observed that minor amounts of the desired product (173) were formed, but not enough to be isolated and characterised.

The next logical progression was to bond cyclopropamitosenes to nitrogen nucleophiles at the C-10 position. This was only partially achieved by following the established methodology by Orelmans *et al.* for mitosenes.[66] In this section our study was concentrated on the reaction of cyclopropamitosene (57) with a variety of nitrogen nucleophiles under reductive conditions, using sodium dithionite. In these cases the reactions were conducted in a mixture of dichloromethane / methanol / water (1:1:1) under nitrogen for a short period of time (varying from 12 min-20 min). The various nucleophiles that were used are as follows: (i) 2-methylaziridine, (ii) *p*-toluidine, (iii) 2-amino-4-hydroxy-6-methylpyrimidine (175) (similar to 2'-deoxyguanosine in structure), as shown below (iv) 2-aminopyrimidine, (v) 2'-deoxyguanosine (177), and (vi) 2'-

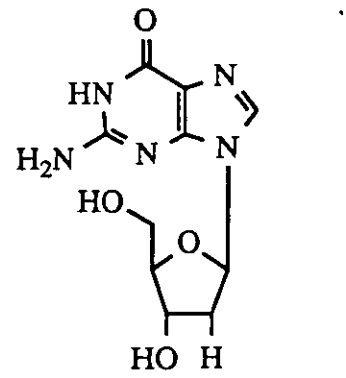
deoxy-3',5'-di-*O*-acetyl guanosine (178). A very promising result was the isolation and characterisation of the C-10 *p*-toluidine substituted product (176), in 53% yield, as illustrated below.



However, these reactions were attempted with only with limited success. The reductive activation reaction failed with nucleophiles such as 2-methylaziridine, 2-amino-4-hydroxy-6-methylpyrimidine (175) as shown below, and 2-aminopyrimidine. If time had permitted, it might have been possible to synthesise the C-10 bond nucleophilic substituted products by altering the reaction conditions, as the reaction with *p*-toluidine worked so successfully.



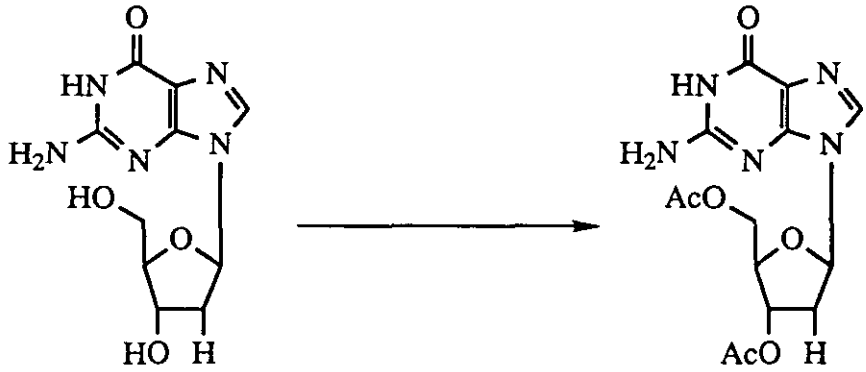
(175)



2'-Deoxyguanosine (177)

The real test for this particular reductive activation reaction of cyclopropamitosene (57) is the reaction with 2'-deoxyguanosine, in the presence of sodium dithionite.[41] This time the reaction conditions were slightly altered. A solution of sodium dithionite in water was added to a mixture of quinone urethane (57), and 2'-deoxyguanosine (177) in dichloromethane / methanol / water (1:1:1), which had previously being purged with nitrogen for 1 hour at 40°C. The mixture was stirred at 40°C for a further 25 minutes. The product was totally water soluble and proved impossible to work with. It was decided then to protect the hydroxy groups of 2'-deoxyguanosine (177) in the hope of making the entire molecule more organic soluble, and hence easier to work with. There are numerous ways in which to protect the hydroxyl groups of 2'-deoxyguanosine (177). We decided to protect the hydroxyl groups as acetyl groups, using two different

procedures. Initially, following the procedure by Li, and Swann,[96] 2'-deoxy-3',5'-di-*O*-acetyl guanosine (178) was isolated (53%), by treatment of 2'-deoxyguanosine (177) with acetic anhydride in pyridine and DMF, at room temperature for 48 hours. Higher yields of 2'-deoxy-3',5'-di-*O*-acetyl guanosine (178) were obtained by an alternative route. In this case, 2'-deoxyguanosine (177) was treated with acetic anhydride, 4-dimethylaminopyridine, triethylamine, in dry acetonitrile at room temperature for 2 hours, to obtain the desired product in a 84% yield, as shown below.[97]



2'-Deoxyguanosine (177) 2'-Deoxy-3',5'-di-*O*-acetyl guanosine (178)

Again using the same experimental procedure as above with 2'-deoxyguanosine (177), but this time using 2'-deoxy-3',5'-di-*O*-acetyl guanosine (178), a mixture was obtained that was soluble in ethanol but unfortunately was too polar to chromatograph on normal silica. Both these compounds proved very difficult to work with, and due to limitation of both time and starting material (57), the reaction could not be repeated.

In Summary: From preliminary experiments it has been shown that C-10 cyclopropamitosene nucleophilic substituted products can be obtained under reductive conditions when potassium ethyl xanthate or *p*-toluidine are used as nucleophiles. The reaction unfortunately, did not work with sodium methoxide, methanol, or a range of other nitrogen nucleophiles. It would be extremely interesting, to vary the reactions conditions and hopefully obtain all the desired nucleophilic substituted products at C-10. It has also been shown in this section that the presence of sodium dithionite is a prerequisite; without this reductant no reaction takes place. All these experimental results indicate that cyclopropamitosenes and mitosenes are reduced in the presence of nucleophiles in a process closely related to the second stage of alkylation in MMC.

CHAPTER 5

ELECTROCHEMICAL MEASUREMENTS AND BIOLOGICAL EVALUATION OF CYCLOPROPAMITOSENES AND MITOSENES

5.1. Electrochemical Measurements of Novel Cyclopropamitosenes and Mitosenes in Non-Aqueous Solution

As discussed in Chapter 1, there is still some controversy in the literature about the oxidation state of the alkylating species formed from MMC, *i.e.* whether the reactive intermediate is the semiquinone radical anion (7) or hydroquinone (2). Nevertheless, the electrochemical properties of the alkylating quinones are very important for their bioreductive activation, either to the semiquinone radical anion or to the hydroquinone. Both the reduction step and subsequent elimination of the leaving group / groups of alkylating quinones are strongly influenced by the substituent attached to the quinone moiety (ring A) : electron-withdrawing substituents facilitate reduction, whereas electron-donating substituents hinder the reduction step. From the onset of this work, the aim of this study was to determine if a correlation could be established between the electrochemical properties of these novel cyclopropamitosenes and mitosenes, the synthesis of which has been discussed in Chapter 2 and 3, with their *in vitro* and *in vivo* cytotoxicity.

The structures of the compounds to be studied were divided into two groups: (i) cyclopropamitosenes - structure [A], and (ii) mitosenes - structure [B], as shown in *Figure 6*. All the structures of these compounds and electrochemical data are listed in *Tables 10-16.*

Relationships between the electrochemical properties and biological cytostatic activity of bioreductive alkylating quinones have been studied mostly using the half-wave potential ($E_{1/2}$) of the quinone reduction step.[98] Hence, our approach was based on this rationale, and we determined E_{redox} values (Redox potentials) *i.e.* E_{redox}, for the one-electron reduction of the quinone moieties to the radical anion. Initially, we determined the E_{redox} (redox potential) in non-aqueous solvents, such as DMF.[99] In dipolar aprotic solvents simple quinones are reduced in two successive steps which are electrochemically reversible under usual voltammetric conditions, as stated in *Equation 2*.[100]

$$Q \underset{}{\overset{e^-}{\rightleftharpoons}} Q^{\cdot-} \overset{e^-}{\rightleftharpoons} Q^{2-} \qquad \textit{(Equation 2)}$$

This important redox system in the mitomycins and related analogues can be studied by cyclic voltammetry. The redox potentials of the cyclopropamitosenes and mitosenes were measured, in a DMF solution using dried tetrabutylammonium tetrafluoroborate (0.1 mol dm^{-3}) as supporting electrolyte. A three-electrode system was employed in which the

working electrode was a platinum flag (1 cm^2). The reference electrode was a sodium chloride saturated calomel electrode (s.s.c.e.) with a platinum-mesh counter electrode. Measurements were conducted in an oxygen freed environment by bubbling solvent-saturated nitrogen through the solution at 25°C prior to running the experiments.[99]

From previous studies with MMC it was shown that the first step of the redox process in *Equation 2* was reversible or at least quasi reversible in DMF. A second electron can be added but at a more negative potential, which was totally irreversible, and this is in contrast to simple quinones.[99] Similar results for the novel cyclopropamitosenes and mitosenes were recorded in our studies and hence we decided only to concentrate on the first electron process, as stated below in *Equation 3*.

$$Q \rightleftharpoons^{e^-} Q^{\cdot-} \qquad \textit{(Equation 3)}$$

Electrochemical measurements for a wide range of cyclopropamitosenes and mitosenes were recorded. Cyclic voltammograms of MMC and cyclopropamitosenes (57), (58), (157) (156), and (155), in DMF at various scan rates (20-400 mVs^{-1}) are illustrated in *Figures 7, 8, 9, 10, 11 and 12* respectively. From the cyclic voltammograms it is apparent that the one-electron reduction process of these compounds are at least quasi reversible with compound (57) being the most reversible. All compounds were run at least in duplicate at various scan rates. On inspection there appears to be little difference between the first and second / third cyclic voltammograms at various scan rates and hence it can be concluded that all the cyclopropamitosenes and mitosenes in this study were stable under these conditions. The lower (more negative) the E_{redox} value, the more difficult it is to reduce the quinone to the semiquinone radical anion. The E_{redox} values were determined with reference to ferrocene (Fc), to avoid any liquid junction potential.

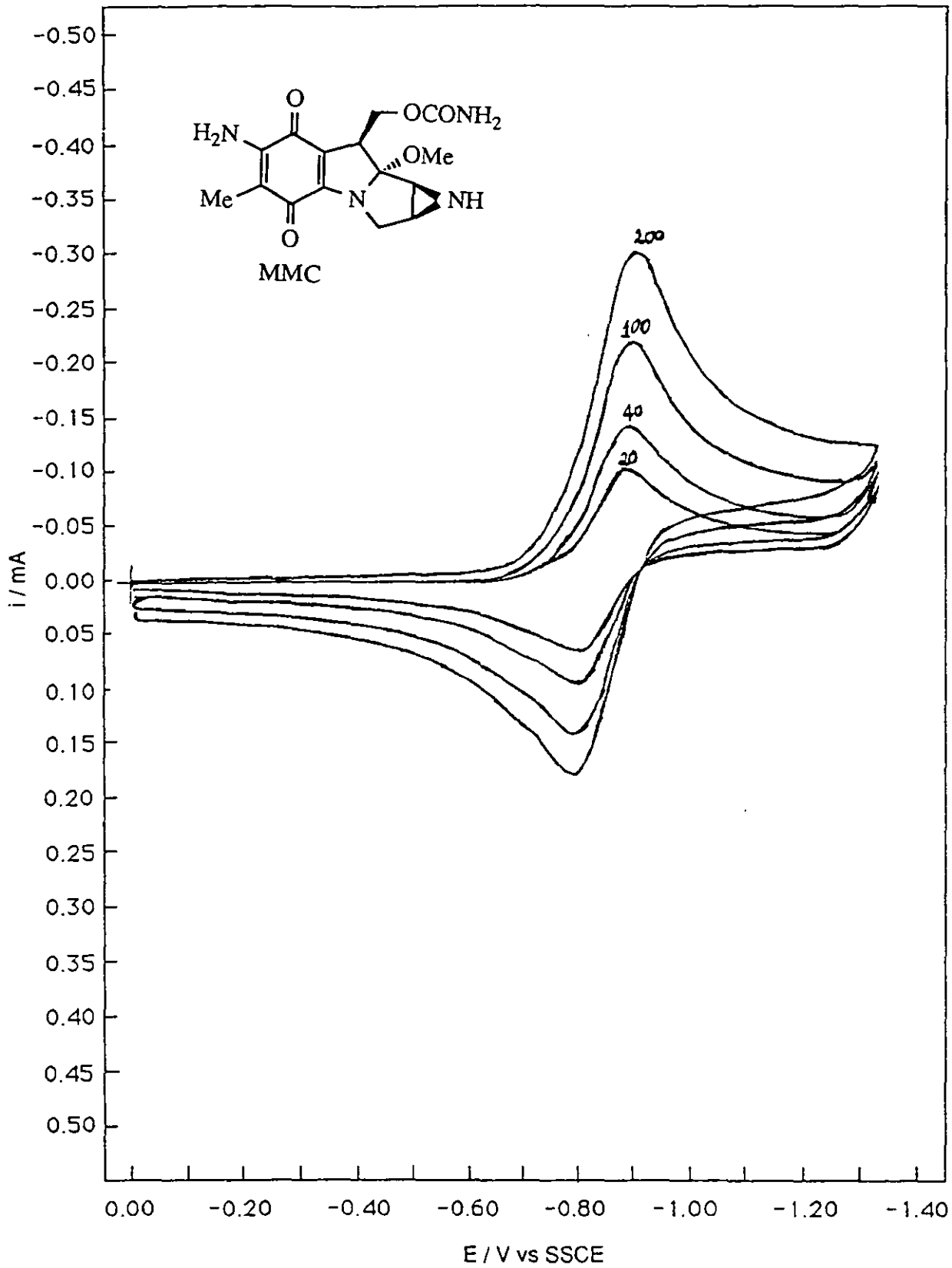


Figure 7: Cyclic voltammogram of MMC in DMF

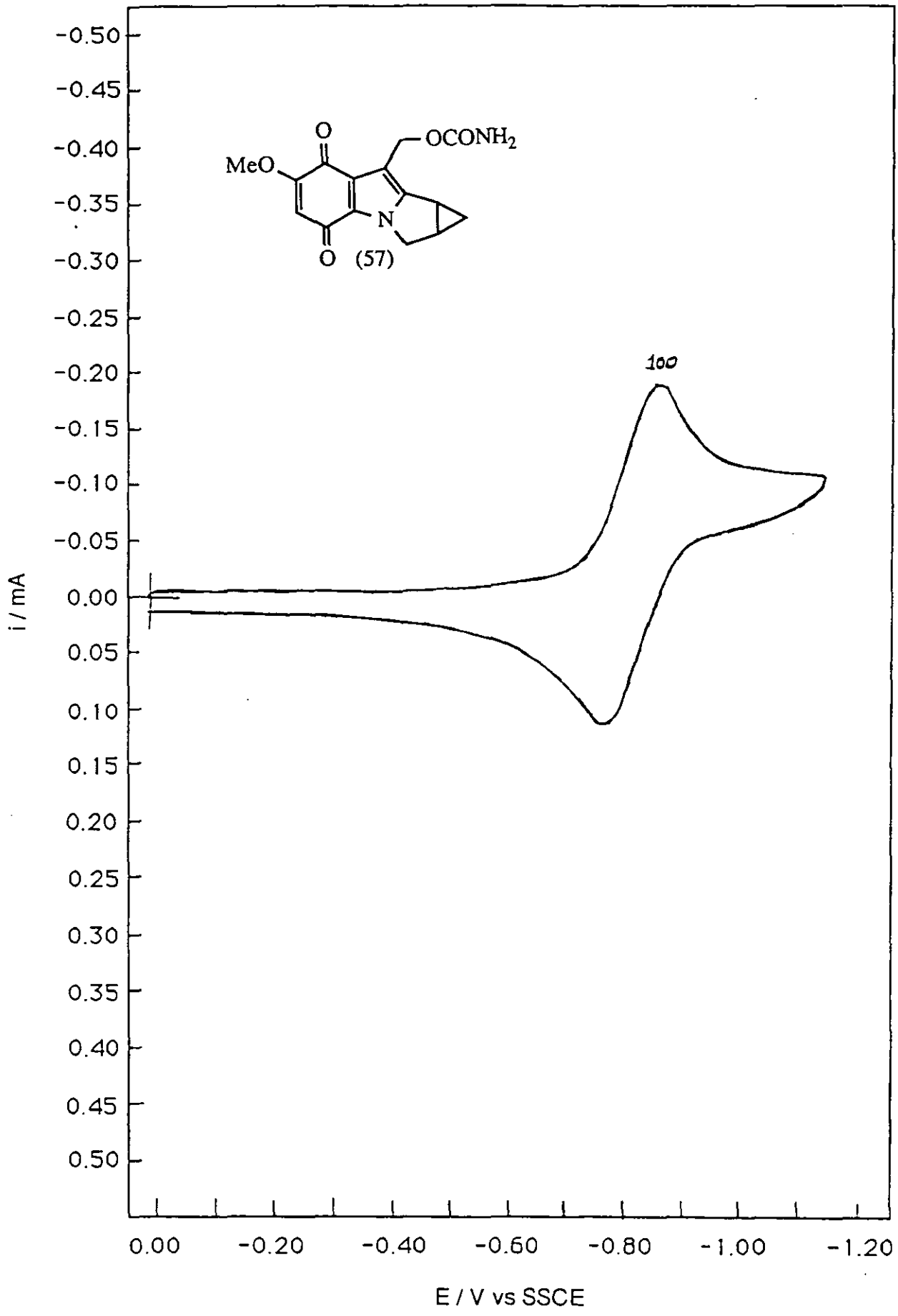


Figure 8: *Cyclic voltammogram of 7-methoxycyclopropamitosene (57) in DMF*

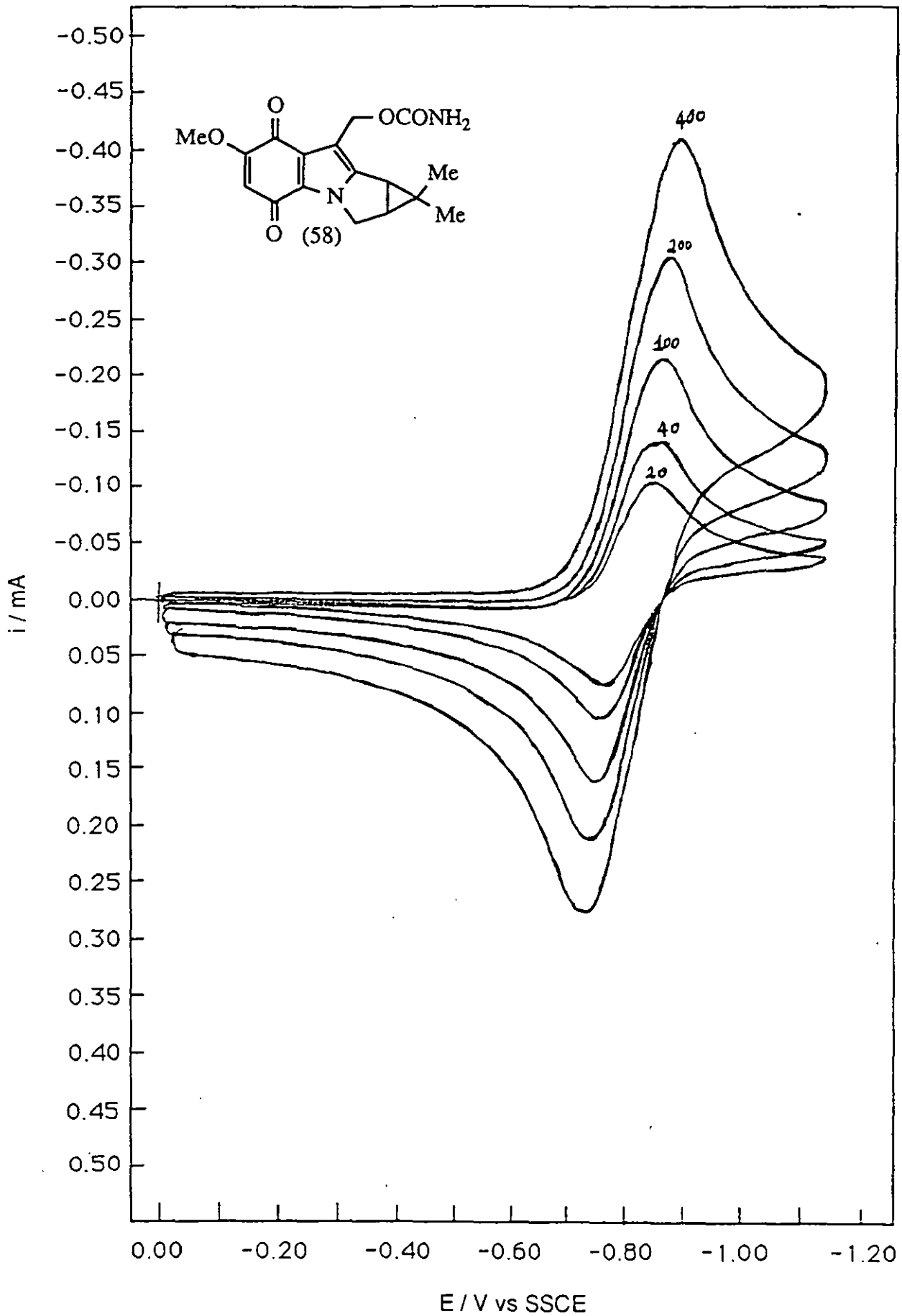


Figure 9: Cyclic voltammogram of (58) in DMF

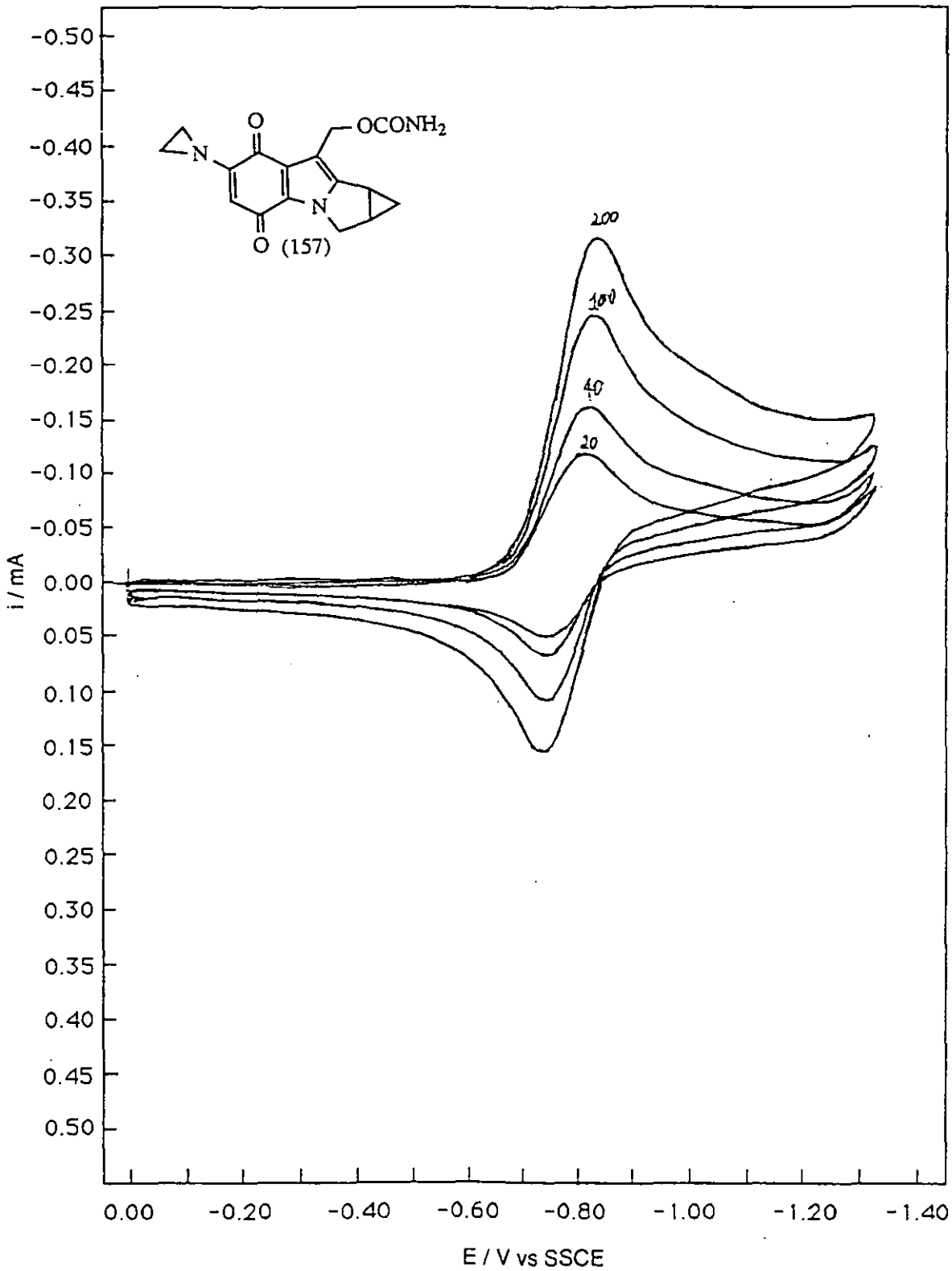


Figure 10: *Cyclic voltammogram of 7-(aziridinyl)cyclopropamitosene (157) in DMF*

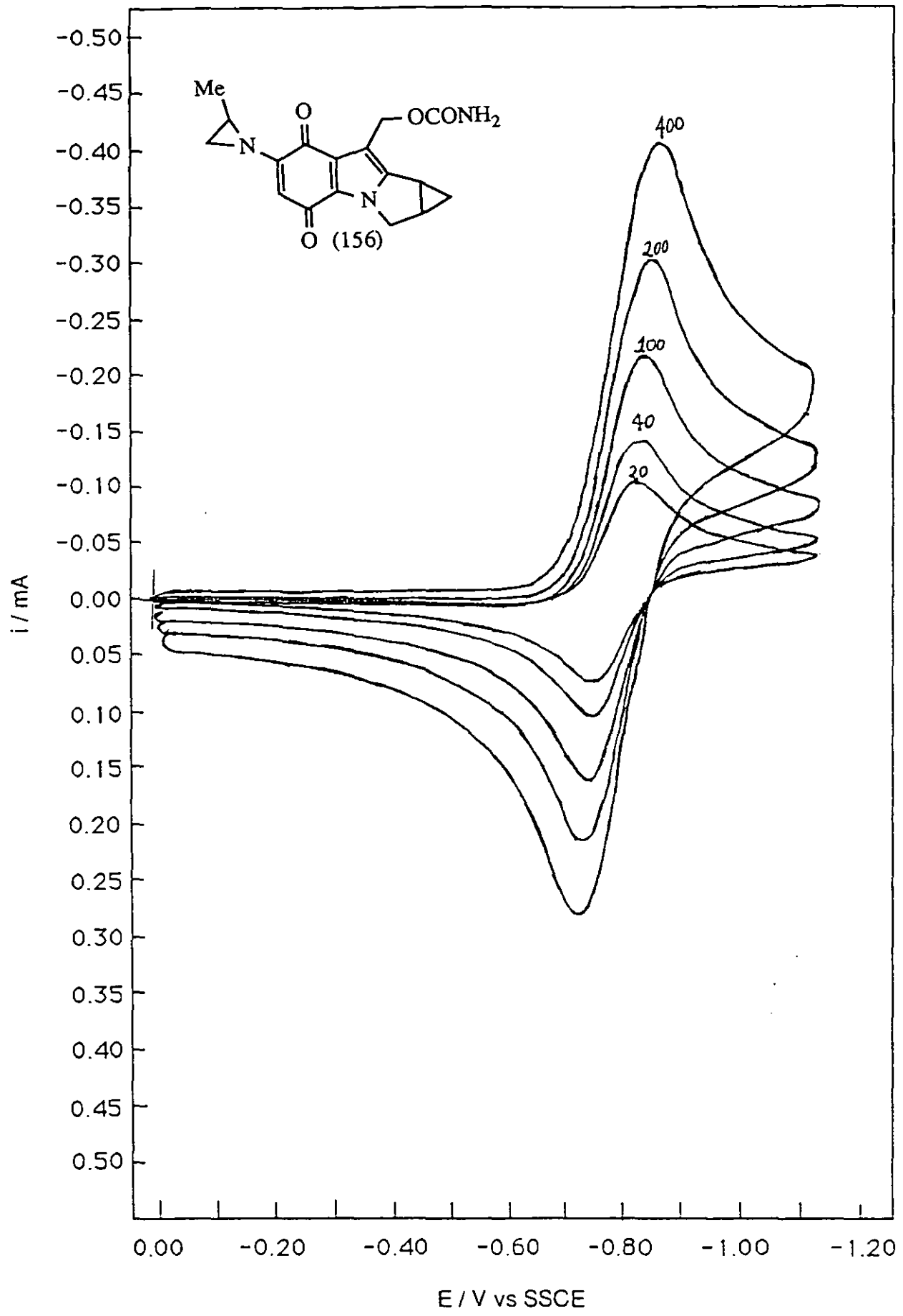


Figure 11: *Cyclic voltammogram of (156) in DMF*

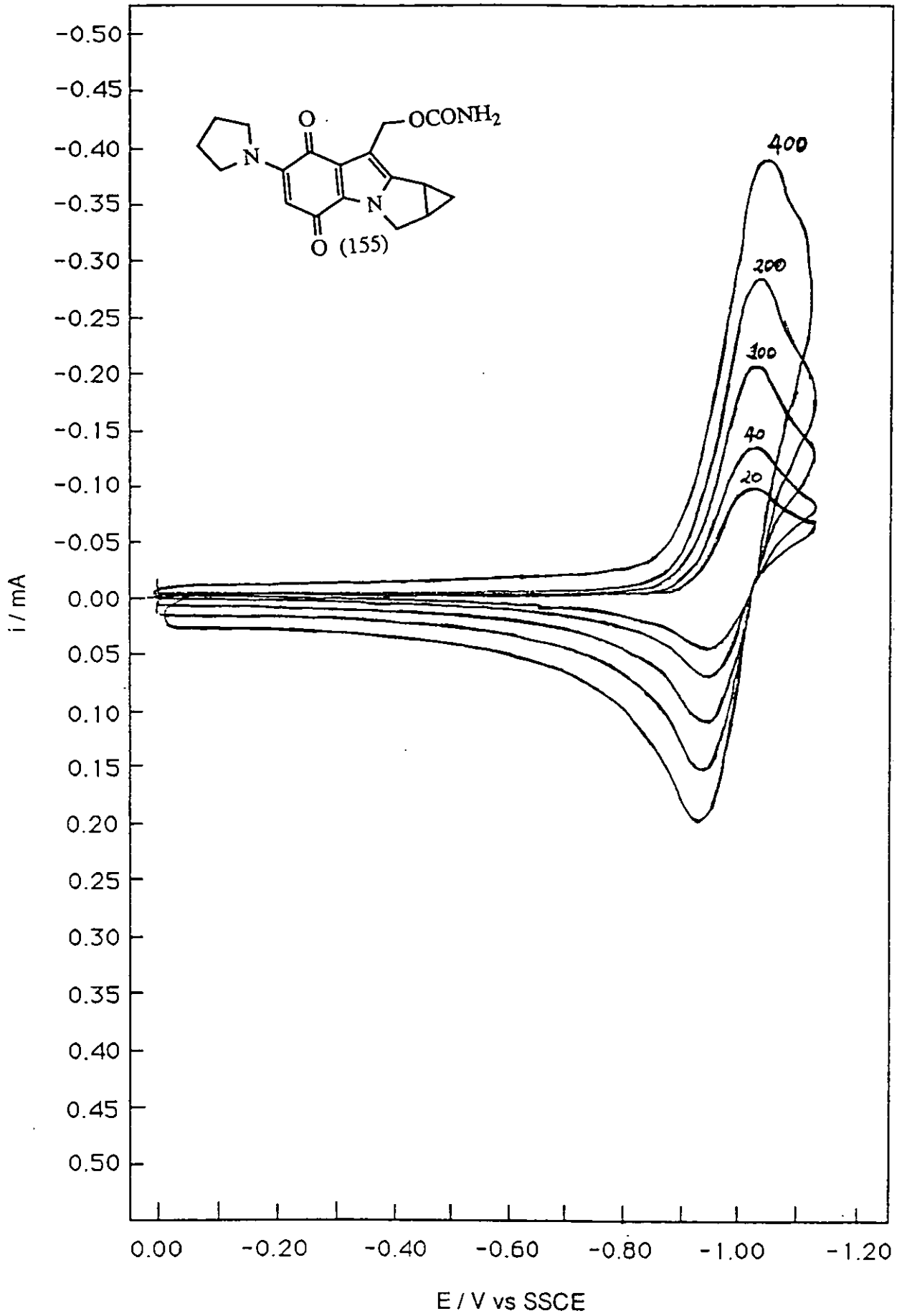
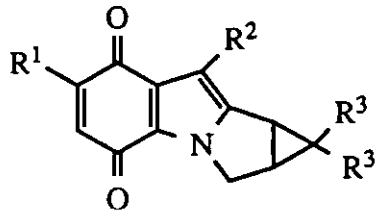
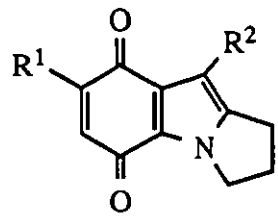


Figure 12: Cyclic voltammogram of (155) in DMF

Our first approach was to establish if a correlation was present between cyclopropamitosene (57) and its analogues, as shown in *Table 10*, with different substituents at the C-7 position, but all having good leaving groups at the C-10 position.



[A]



[B]

***Figure 6:** Basic structures of Cyclopropamitosene [A] and Mitosene [B]*

Table 10

Compound	Structure	R^1	R^2	R^3	Ered. / V
(157)	[A]	Aziridinyl	CH_2OCONH_2	H	-1.360
(156)	[A]	2-Methylaziridinyl	CH_2OCONH_2	H	-1.371
(90)	[A]	MeO	$OCOCH_3$	H	-1.380
(145)	[A]	$MeOCH_2CH_2O$	CH_2OCONH_2	H	-1.390
(57)	[A]	MeO	CH_2OCONH_2	H	-1.395
(154)	[A]	$MeOCH_2CH_2NH$	CH_2OCONH_2	H	-1.552
(155)	[A]	Pyrrolidinyl	CH_2OCONH_2	H	-1.572

E_{redox} of MMC = -1.421 V (Fc)
E_{redox} of MMC = -0.88 V (lit.[99] -0.92 V) (s.s.c.e.)

Where: E_{redox} = Redox Potential
Fc = Ferrocence

The most striking observation from *Table 10* is that even small structural modifications between compounds resulted in large differences in their redox potential. From *Table 10*, a trend correlating increasing electron donating power of substituents at C-7 on ring A with more negative values for redox potentials was observed. Comparing compounds (57) with (155), clearly the pyrrolidinyl group is a better donating group than the 7-methoxy substituent of compound (57), thus recording a lower value for its redox potential, -1.572 V. This time comparing (157) and (57), the lone pair of electrons on the nitrogen on the aziridine ring cannot be delocalised into the quinone moiety due to ring strain (as previously discussed in Chapter 3), and hence this compound has a more positive redox potential value of -1.360 V. Another interesting feature of *Table 10* is the 2-methylaziridinyl analogue (156). The methyl group at the 2-position of compound

(156) has the effect of pushing more electron density into the quinone moiety, thus recording a more negative value of -1.371 V in comparison to the analogous aziridine derivative (157). Taking the MMC value as a reference (-1.421 V), one can see that only considering redox potential, the aziridinyl derivatives and alkoxide analogues are easier to reduce than MMC. However, substituents at position 7 with better electron donating groups such as pyrrolidinyl and 2-methoxyethylamino are more difficult to reduce from the quinone to the semiquinone radical anion, and thus recording a more negative value for its redox potential. In a previous study by Andrews *et al.*, [99] a redox potential of -0.92 V (s.s.c.e.) was recorded. In our experiment a redox potential of -0.88 V (s.s.c.e.) was recorded. These results are comparable. In our experiments ferrocene was used as an internal reference to avoid any liquid junction potential whereas the other group assumed the junction potential to be zero.[99] These trends were also observed in other series as illustrated below in *Tables 11, 12, 13, and 14.*

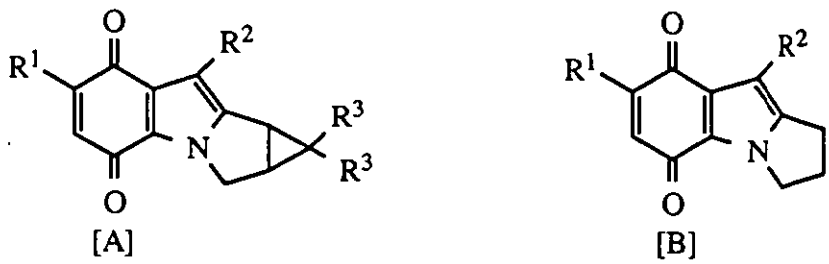


Figure 6: *Basic structures of Cyclopropamitosene [A] and Mitosene [B]*

Table 11

Compound No.	Structure	R^1	R^2	R^3	Ered. / V
(158)	[A]	Aziridinyl	CH_2OCONH_2	Me	-1.355
(58)	[A]	MeO	CH_2OCONH_2	Me	-1.370
(159)	[A]	Pyrrolidinyl	CH_2OCONH_2	Me	-1.588

E_{redox} of MMC = -1.421 V (Fc) **Where:** E_{redox} = Redox Potential

E_{redox} of MMC = -0.88 V (lit.[99] -0.92 V) (s.s.c.e.) Fc = Ferrocene

Table 12

Compound No.	Structure	R^1	R^2	R^3	Ered. / V
(160)	[A]	Aziridinyl	H	H	-1.384
(161)	[A]	2-Methylaziridinyl	H	H	-1.401
(78)	[A]	MeO	H	H	-1.403

Table 13

Compound No.	Structure	R^1	R^2	R^3	Ered. / V
(162)	[B]	Aziridinyl	CH_2OCONH_2	-	-1.385
(163)	[B]	2-Methylaziridinyl	CH_2OCONH_2	-	-1.395

Table 14

Compound No	Structure	R^1	R^2	R^3	Ered. / V
(164)	[B]	Aziridinyl	H	-	-1.398
(133)	[B]	MeO	H	-	-1.412

Table 15

Compound No.	Structure	R^1	R^2	R^3	Ered. / V
(58)	[A]	MeO	CH_2OCONH_2	Me	-1.370
(90)	[A]	MeO	$OCOCH_3$	H	-1.380
(139)	[B]	MeO	$OCOCH_3$	-	-1.387
(145)	[A]	$MeOCH_2CH_2O$	CH_2OCONH_2	H	-1.390
(57)	[A]	MeO	CH_2OCONH_2	H	-1.395
(78)	[A]	MeO	H	H	-1.403
(133)	[B]	MeO	H	-	-1.412

E_{redox} of MMC = -1.421 V (Fc) **Where:** E_{redox} = Redox Potential

E_{redox} of MMC = -0.88 V (lit.[99] -0.92 V) (s.s.c.e.) Fc = Ferrocene

All the cyclopropamitosenes and mitosenes in *Table 15* possess a C-7 alkoxide substituent. From the Table, one can see a trend correlating good leaving groups at the C-10 position with more positive redox potentials values *i.e.* the compounds become easier to reduce. Compounds with good leaving groups, such as acetoxy groups for both the cyclopropamitosene (90) and mitosene (139) series recorded a more positive value for its redox potential, -1.380 V and -1.387 V respectively, in comparison to the parent urethane (57). Also, compounds with no leaving group at the C-10 position, such as (78) and (133) are the most difficult to reduce, thus recording a more negative redox potential value, -1.403 V, and -1.412 V respectively, in comparison to all the other above compounds in *Table 15*. As illustrated previously, small structural changes can dramatically alter the redox potential. This point is again evident from comparing compounds with different substituents at the C-1a position, and compounds without the cyclopropane ring. When the two hydrogens of compound (57) are replaced by two methyl groups to give compound (58), a more positive redox potential value of -1.370 V was recorded. However, when the cyclopropane ring has been removed, a lower redox potential value was recorded, as these compounds become more difficult to reduce. These trends are also reflected in an alternative series, the 7-aziridinyl series as illustrated below in *Table 16*.

Table 16

Compound No.	Structure	R^1	R^2	R^3	Ered. / V
(158)	[A]	Aziridinyl	CH_2OCONH_2	Me	-1.355
(157)	[A]	Aziridinyl	CH_2OCONH_2	H	-1.360
(162)	[B]	Aziridinyl	CH_2OCONH_2	-	-1.385
(160)	[A]	Aziridinyl	H	H	-1.384
(164)	[B]	Aziridinyl	H	-	-1.398

E_{redox} of MMC = -1.421 V (Fc) **Where:** E_{redox} = Redox Potential

E_{redox} of MMC = -0.88 V (lit.[99] -0.92 V) (s.s.c.e.) Fc = Ferrocene

In Summary : Electrochemical parameters were recorded for a wide range of synthesised cyclopropamitosenes and mitosenes. Some trends for different series were observed, and the conclusions are summarised below;

(i) There is a clear tendency for inductive electron withdrawing substituents such as

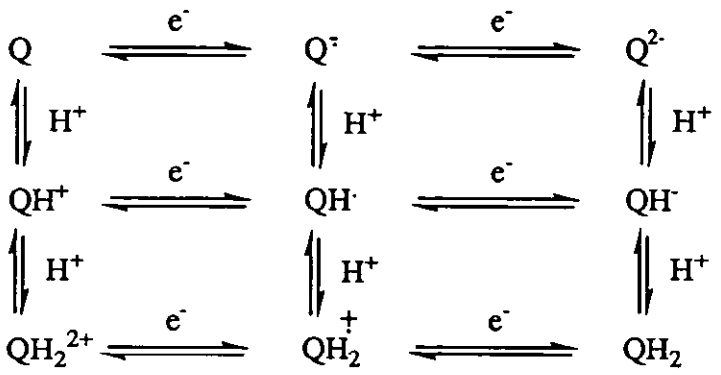
aziridinyl or 2-methylaziridinyl groups at the C-7 position to facilitate the one electron reversible reduction process.
(ii) Derivatives with the methyl group at the 2-position of aziridine were more difficult to reduce than the analogous aziridine derivatives.
(iii) Analogues of both cyclopropamitosenes and mitosenes with good leaving groups at the C-10 position, such as acetoxy groups facilitate the reduction process, whereas derivatives with no leaving groups were more difficult to reduce.
(iv) The presence of the cyclopropane ring especially with substituents at the C-1a position also appears to facilitate the reduction process.

With these trends in mind, it would be interesting to establish if a correlation between the electrochemical parameters of these novel cyclopropamitosenes and mitosenes with their *in vitro / in vivo* activity exists. If a useful correlation could be found then electrochemical measurements might be used as a screen for the biological activity of these alkylating quinones.

5.1.1. Electrochemical Studies in an Aqueous Solution

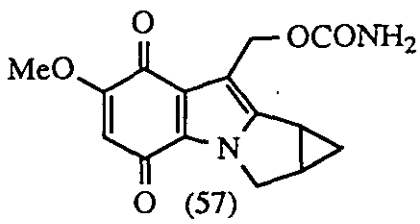
The above experiments were conducted in DMF, and hence we decided to conduct the experiments under physiological conditions to investigate if the trends discussed above are apparent in both solvent systems. This time the cathodic peak potential ($E_{p.c}$) was measured in a mixture of DMF and phosphate buffer at a pH of 7.4 using cyclic voltammetry. A three-electrode system was employed in which the working electrode was a hanging mercury drop electrode (Metrohm). The reference electrode was a sodium chloride saturated calomel electrode (s.s.c.e.). No internal reference was needed in these experiments.[101]

In the aqueous situation, all the possible protonated forms of *Equation 2* must be considered, and a nine species array of electrochemical pathways is possible as stated in *Equation 4*.[100]



(Equation 4)

Electrochemical measurements for a range of cyclopropamitosenes and MMC were recorded. Cyclic voltammograms of two of these compounds, 7-methoxycyclopropamitosene (57) and (78) in a mixture of DMF and phosphate buffer at 100 mVs^{-1} are illustrated in *Figures 13, and 14* respectively. There is one immediate striking observation and that is that the two cyclic voltammograms appear totally different. Initially, the cyclic voltammogram of 7-methoxycyclopropamitosene (57) will be discussed. The initial electrochemical step responsible for peak A is probably due to the reversible electrochemical two electron and two proton reduction (as discussed later) to the hydroquinone which is coupled to complex irreversible chemical steps(s). In the higher scan rates of multicycle voltammetry the cyclic voltammogram appears reversible. It can be concluded from this that at higher scan rates (500 mVs^{-1}) the electrochemical process is occuring so quickly and hence the chemical step is not observed. Unfortunately, in this case the cyclic voltammogram is not capable of providing information on the chemical step. The cyclic voltammogram of compound (78) is totally reversible, with a cathodic peak potential of -455 mV (s.s.c.e.). This is only an electrochemical process *i.e.* probably reduction to a semiquinone radical anion and no chemical reactions occurs. This is in agreement with the fact that this molecule has no C-10 alkylation site. All the data from the cyclic voltammograms of MMC and five cyclopropamitosenes are summarised in *Table 17.*



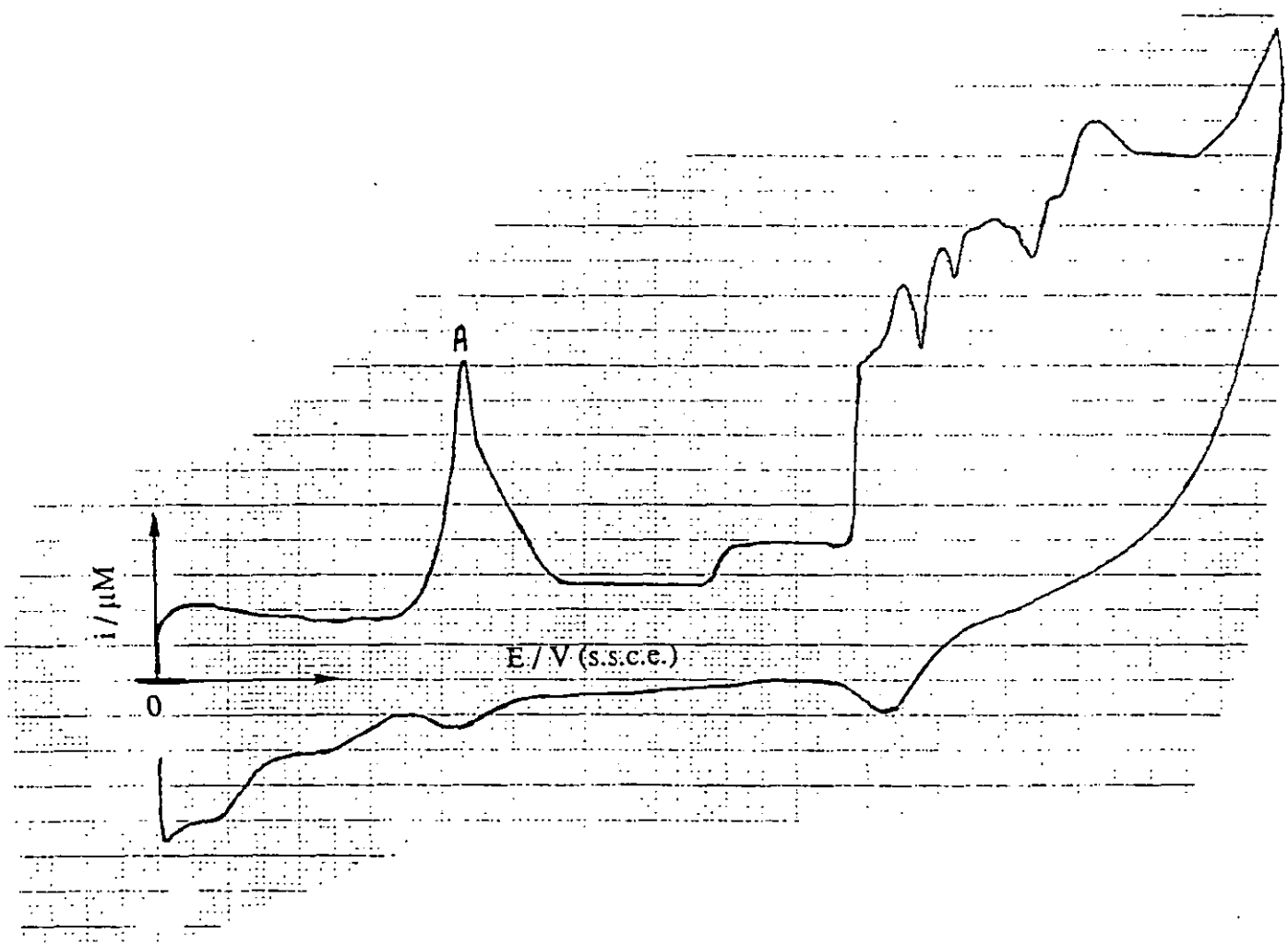
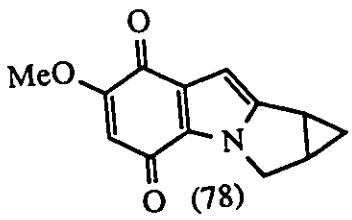


Figure 13: *Cyclic voltammogram of (57) in a mixture of DMF and phosphate buffer solution (pH 7.4)*



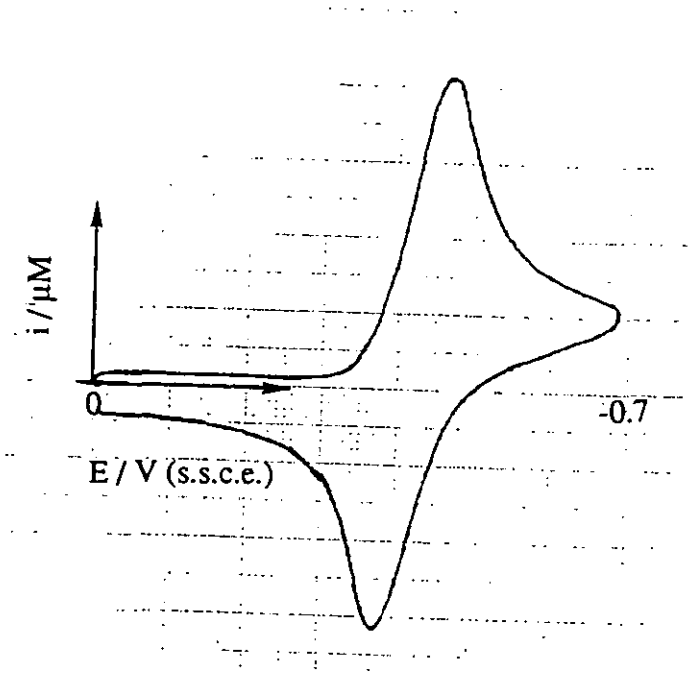


Figure 14: *Cyclic voltammogram of (78) in a mixture of DMF and phosphate buffer solution (pH 7.4)*

Table 17

Compound Number	$E_{p.c}$ / mV (s.s.c.e.)	$(E_{p.c} - E_{p/2})$ mV	No. of electrons involved in the redox process	$\frac{i_{p.a}}{i_{p.c}}$
(57)	-425	25	2	0.52 not reversible
(90)	-480	115	-	-
(155)	-525	45	1 or 2	0.44 not reversible
(161)	-420	55	1	1.09 reversible
(78)	-455	55	1	1.04 reversible
MMC	-455	45	2	0.87 not reversible
Benzoquinone	-10	50	1	0.98 reversible

$E_{p.c}$ = Cathodic peak potential *i.e.* one or two electron reduction of the quinone moieties to the semiquinone radical anion or hydroquinone

$E_{p/2}$ = Half cathodic peak potential; $E_{p/2} = E_{1/2} + 28 / n$ mV at 25°C

$(E_{p.c} - E_{p/2}) = 56.2 / n$ mV at 25°C [When $(E_{p.c} - E_{p/2}) = 56.2$ mV, 1 electron]

Where: $E_{1/2}$ = Half wave potential, $i_{p.a}$ = Anodic peak current, $i_{p.c}$ = Cathodic peak current, and n = Number of electrons

From the Table, it can be concluded that the same trends hold as in the DMF experiments. Comparing compounds (57) and (155), clearly the 2-methoxyethylamino group is a better donating group than the 7-methoxy substituent of compound (57), thus recording a lower value for its cathodic peak potential, -525 mV (s.s.c.e.). Inductive electron withdrawing substituents at the C-7 position such as an aziridinyl group in compound (161) facilitate the reduction process thus recording a more positive cathodic peak potential in comparsion to the parent analogue (78). From the Table, one can see a trend correlating good leaving groups, such as acetoxy and urethane groups at the C-10 position with the ease of reduction. However, in this study compound (90) is more difficult to reduce with a cathodic peak potential of -480 mV in comparsion to the analogous urethane compound

(57) (-425 mV). This result is in contrast to the DMF experiments. Compounds with no leaving group at the C-10 position, such as (161) and (78) are the most difficult to reduce, recording more negative redox potential, -420 mV and-455 mV respectively. In conclusion, the same trends are observed in non-aqueous and aqueous buffer solution (pH 7.4). The cathodic peak potential of MMC in this assay was-455 mV (s.s.c.e.) or -450 mV (s.c.e.). This value is close to the value reported in the literature by Rao *et al* [-383 mV (s.c.e.)].[101]

However, considerably more information can be gained from *Table 17*. $E_{p.c} - E_{p/2}$ indicates the number of electrons transferred during the reduction process [one electron reduction occurs when $(E_{p.c} - E_{p/2}) = 56.2$ mV]. From the Table, it is suggested that 7-methoxycyclopropamitosene (57) is probably reduced to the hydroquinone, whereas compounds (161) and (78) are reduced to the semiquinone radical anion. In a study Rao *et al.* calculated the difference $(E_{p.c} - E_{p/2})$ to be 40-50 mV (varying a little over difference scan rates). The authors concluded that the mechanism of reduction of MMC belongs to the ECE (electrochemical - chemical - electrochemical) class, where C represents one or more steps. The electrochemical steps are considered to be reversible and the chemical step are irreversible.[101] In our experiments $(E_{p.c} - E_{p/2})$ was calculated to be 45 mV. It is extremely difficult to state whether compound (155) are reduced to the semiquinone radical anion or hydroquinone as it has a $(E_{p.c} - E_{p/2}) = 45$ mV. Unfortunately, with 7-methoxycyclopropamitosene (57) this is a very controversial result as it appears to disagree with some of the biological results. The biological results which are in disagreement with the electrochemistry are firstly on addition of dicoumarol to V79 cells during hypoxic exposure to (57) no change in IC_{50} was observed. This suggests that DT-diaphorase (an obligate 2-electron enzyme) probably is not important with regard to the activation of 7-methoxycyclopropamitosene (57) in air. From cell lines experiments it is suggested that DT-diaphorase probably plays no role in the activation of 7-methoxycyclopropamitosene (57). This compound (57) may probably be metabolised by NADPH : cytochrome P450 reductase (which is a one electron enzyme) as discussed in next Section. 7-Methoxycyclopropamitosene (57) may not be a substrate for DT-diaphorase but it might be for other two electron reductases, and hence then this would then agree with the electrochemical findings. It is extremely complicated and requires further investigation. Workman *et al.* in the near future will hopefully confirm if 7-methoxycyclopropamitosene (57) is a substrate or not for DT-diaphorase. The most striking observations from these results is that even small structural modifications between compounds may result in totally diferent mode of action of these cyclopropamitosene *i.e.* whether the reactive species is the semiquinone radical anion or

hydroquinone.

In Summary: Electrochemical parameters both in DMF and an aqueous buffer solution at pH 7.4 were recorded for a range of synthesised cyclopropamitosenes and mitosenes (DMF experiments only). The same trends were observed as discussed in summary 1 above for the two sets of different experiments. From the hanging mercury drop electrode experiment, the result suggests that 7-methoxycyclopropamitosene (57) is reduced to the hydroquinone which is coupled to chemical step(s). This is in contrast to compounds (78) and (161) which are suggested to be reversibly reduced to the semiquinone radical anion. In the case with 7-methoxycyclopropamitosene (57) further investigation is necessary to figure out the discrepancies between the electrochemical and biological results with regard to the mode of action.

5.2. Biological Evaluation of the Novel Cyclopropamitosenes and Mitosenes

5.2.1. Introduction To Bioreductive Drugs

Bioreductive drugs are defined as compounds that are selectively toxic to hypoxic tumour cells. These bioreductive drugs, can selectively target hypoxic tumour populations that are resistant to both radiation and to drug based therapies. This selective hypoxic cell cytotoxicity is due, amongst other factors, to the ability of an enzyme to metabolise these drugs to a toxic species under hypoxic conditions. Many enzymes have been implicated in this reaction, including cytochrome P450, NADPH : cytochrome P450 reductase, xanthine oxidase, aldehyde oxidase, and DT-diaphorase. However, it is important to remember that species differences and multiple isoforms exist for some of these enzymes. The ease of bioreduction of a given drug will depend upon the ability of the drug to act as a substrate for the intracellular reductase(s) and the expression of these enzyme(s) within the particular cell type. Furthermore, depending upon whether or not the bioreductive drug is reduced in a one or two electron step, the initial step in the reduction may be reversed by oxygen, as discussed in Chapter 1. These factors all contribute to the variability of cytotoxic efficiency observed in different cell types and also the variable magnitude of the hypoxic / oxic cytoxic differential.

The enzymology contributing to activation of MMC under aerobic and hypoxic conditions

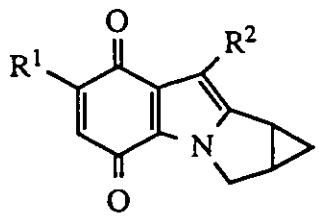
is not well defined. MMC is preferentially toxic to hypoxic tumour cell both *in vitro* and *in vivo*. The activity of MMC is highly dependent on its environment *i.e.* MMC activity increases at low oxygen concentration and low pH. Oxygen has been suggested to limit the cytoxicity of the semiquinone radical (7) derived from MMC by a one electron reduction process. Under aerobic conditions the semiquinone redox cycles to generate oxygen radicals which are relatively non-toxic. Some DNA strand breaks is achieved by the highly oxidising hydroxy radicals (superoxides and hydrogen peroxide), but the effect of these radicals is diminished by enzymes such as superoxide dismutase. However, under hypoxic conditions the semiquinone radical anion (7) may alkylate, either directly or *via* the hydroquinone (2) formed by disproportionation, leading to increased cytoxicity. At the present moment, there is still controversy over the reactive intermediate of MMC that alkylates to DNA, whether or not it is the semiquinone radical anion (7) or the hydroquinone (2). As stated earlier several enzymes can reductively activate MMC but the role of NADPH : cytochrome P450 reductase, and more controversially DT-diaphorase in clinical material is unclear.

Hence, at present, bioreductive drugs are likely to be used in a number of different ways. Firstly, specifically to kill hypoxia cells, taking advantage of the presence of hypoxia for the activation of drugs to cytoxic metabolites. Secondly, to exploit differences in the levels of reductases in different cell types in order to direct appropriate drugs to particular human tumours based on their enzymology.

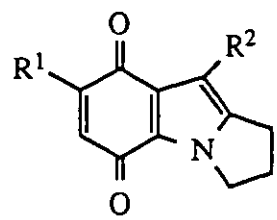
5.2.2. Biological Activity

The aims of this study were to determine the antitumour activity both *in vitro* and / or *in vivo* for a series of novel cyclopropamitosenes and mitosenes, the synthesis of which has been previously discussed. It was also hoped that a correlation between potency and reduction potential could be established. The biological experiments were carried out under the direction of Dr Ian Stratford at the MRC Radiobiology Unit.

Initially, the *in vitro* activity of a series of cyclopropamitosenes and mitosenes was determined on V79 cell types both under oxic and hypoxic conditions, as below shown in *Figures 15, 16, 17* and *Table 18*. The *in vitro* activities are represented as IC_{50} value, indicating the concentration (μM) of drug that inhibits survival by 50% following exposure to cells for 3 hours at 37°C. The MTT assay was used to estimate toxicity in these experiments. The structure of the various novel cyclopropamitosenes and mitosenes are illustrated in *Figure 6*.



[A]



[B]

Figure 6: Basic Structure of Cyclopropamitosene [A], and Mitosene [B]

Figure 15: Mitomycin C, EO9, 7-Methoxycyclopropamitosene (57) and 7-(Aziridin-1-yl)cyclopropamitosene (157) Aerobic Toxicity in V79 Cells

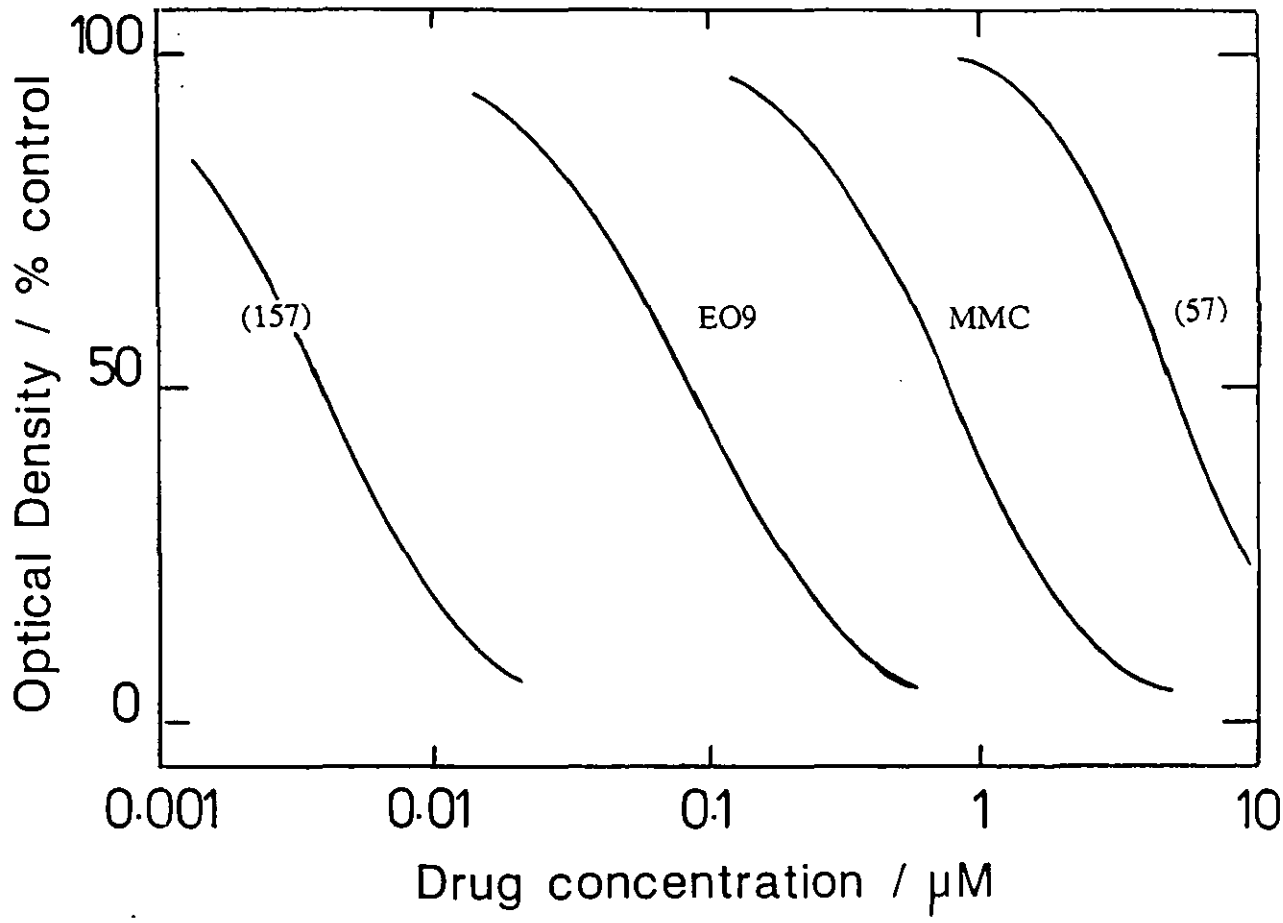
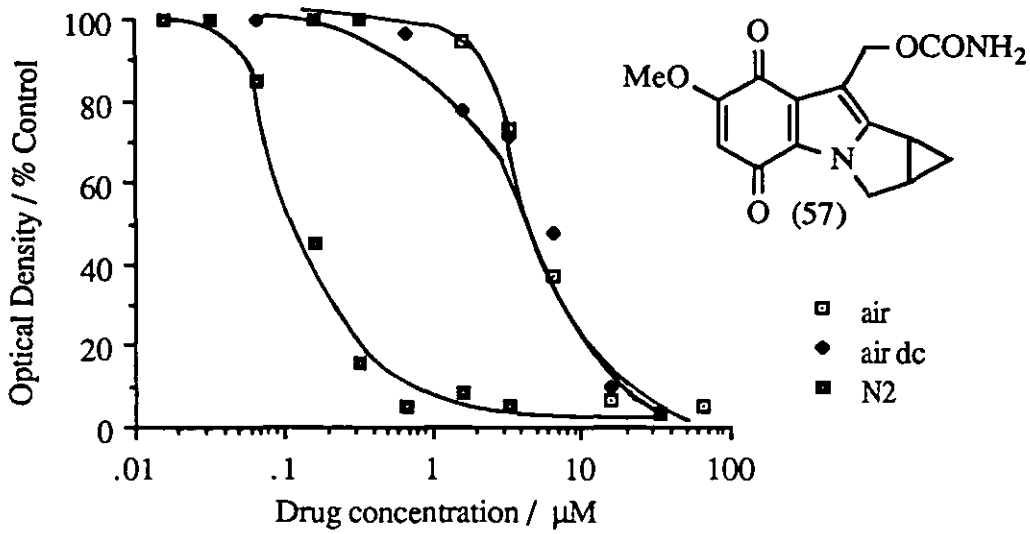
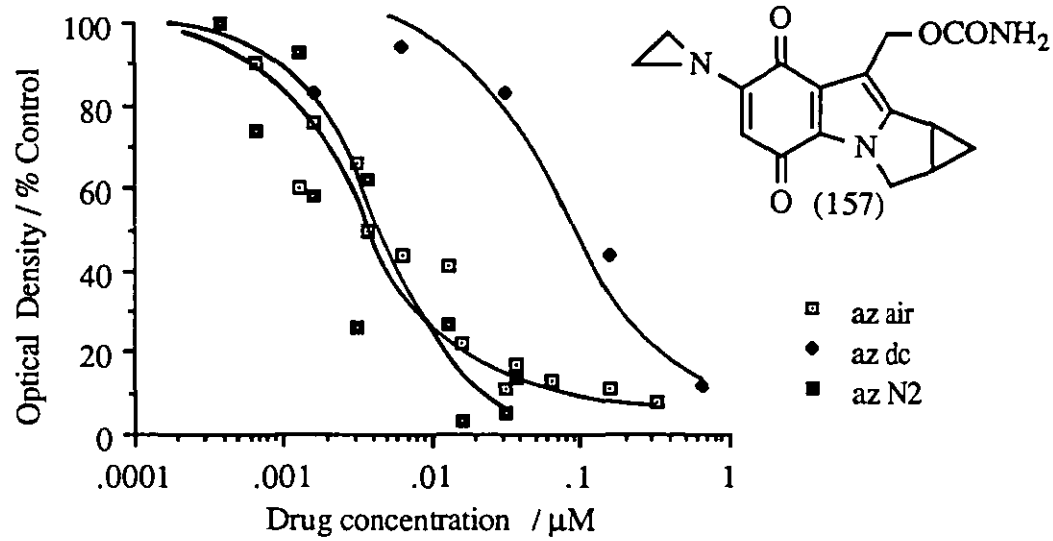


Figure 16: 7-Methoxycyclopropamitosene (57) In Vitro Aerobic and Anaerobic Toxicity in V79 Cells



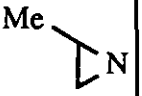
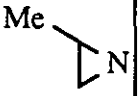
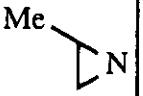
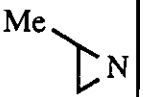
Where: dc = dicoumarol

Figure 17: 7-(Aziridin-1-yl)cyclopropamitosene (157) In Vitro Aerobic and Anaerobic Toxicity in V79 Cells



Where: dc = dicoumarol az = 7-(aziridin-1-yl)cyclopropamitosene (157)

Table 18

Compound No.	Structure	R^1	R^2	$IC_{50}(\mu M)$ Air	N_2	Air/N_2
(157)	[A]		CH_2OCONH_2	0.003	0.003	1.0
(156)	[A]		CH_2OCONH_2	1.2	0.055	22
(57)	[A]	OMe	CH_2OCONH_2	4.8	0.14	34
(58)*	[A]	OMe	CH_2OCONH_2	3.0	0.12	25
(90)	[A]	OMe	CH_2OCOMe	12	1.2	10
(155)	[A]		CH_2OCONH_2	140	140	1.0
(160)	[A]		H	7.0	2.0	3.5
(161)	[A]		H	90	35	25
(78)	[A]	OMe	H	200	200	1.0
(162)	[B]		CH_2OCONH_2	0.07	0.005	14
(163)	[B]		CH_2OCONH_2	0.2	0.07	3.5
(138)	[B]	OMe	CH_2OCONH_2	6.0	0.1	60
(139)	[B]	OMe	CH_2OCOMe	11	0.45	24
(164)	[B]		H	2.2	1.1	2.0
(165)	[B]		H	80	4.0	20
(133)	[B]	OMe	H	300	300	1.0

Note: Compund (58)* has two methyl groups as R^3 groups

The results from *Figures 15, 16, 17* and *Table 18* are quite striking, and show that even small structural modifications resulted in very significant differences in *in vitro* activity of these compounds. Some of these compounds showed better *in vitro* activity than the

lead compound, MMC. Air toxicity due to modification at the C-7 position is always in the order of the aziridinyl, 2-methylaziridinyl, and methoxy groups, with the aziridinyl compounds being the most potent. Also from *Table 18*, it can be seen that compounds with 7-methoxy group and C-10 urethane such as (57), (58), and (138) always have a large oxic / hypoxic differential. However, it is not possible to predict the oxic / hypoxic differential for the aziridinyl and 2-methylaziridinyl compounds as shown in *Table 18*. *i.e.* compound (157) has a differential of 1 and (156) has a differential of 22, whereas in the mitosenes (162) and (163) the situation is reversed. The magnitude of the oxic / hypoxic differential, however, is as yet difficult to predict just from the structure of the compound.

The difference in potency in air between compounds, 7-methoxycyclopropamitosene (57), 7-(aziridin-1-yl)cyclopropamitosene (157), MMC and EO9 are illustrated in *Figure 15*. Clearly, compound (157) is the most effective agent and is active in the nM range.

7-Methoxycyclopropamitosene (57) has only one alkylation site at C-10 and the proposed mode of action closely resembles the second stage of alkylation of DNA by MMC, as previously discussed in Chapter 4. However, 7-(aziridin-1-yl)cyclopropamitosene (157) now has two potential alkylation sites; (i) the C-10 position and (ii) opening of the aziridine ring after bioreductive activation either to the semiquinone radical anion or hydroquinone. *Figures 16, and 17* shows the difference in potency between, 7-methoxycyclopropamitosene (57), and 7-(aziridin-1-yl)cyclopropamitosene (157) on V79 cell lines under aerobic and anaerobic conditions respectively. 7-Methoxycyclopropamitosene (57) shows a much greater specificity for hypoxic cells compared with that of MMC, as shown in *Figure 16*. This is illustrated by the fact that (i) 7-methoxycyclopropamitosene (57) is considerably more active than MMC under hypoxic conditions, and (ii) the hypoxic / oxic differential of 34 for 7-methoxycyclopropamitosene (57) is much greater than that of MMC, which is only 2. This result is very promising, and the compound is undergoing further investigation at the present time. When dicoumarol (an inhibitor of DT-diaphorase) was added to the V79 cells during hypoxic exposure to compound (57), no change in IC_{50} value was observed as shown above in *Figure 16* (3 μM in both cases). Since no difference in IC_{50} value is observed, which suggests that DT-diaphorase may not be important with regard to the activation of 7-methoxycyclopropamitosene (57) in air. Unfortunately, as discussed in Section 5.1.1. this may contradict the electrochemical results, which suggest that the hydroquinone is the reactive intermediate.

7-(Aziridin-1-yl)cyclopropamitosene (157) is equally effective in killing both aerobic and

hypoxic cells at extremely low concentration, as shown in *Figure 17*. When dicoumarol was added to the V79 cells during hypoxic exposure to 7-(aziridin-1-yl)cyclopropamitosene (157) the IC_{50} was no longer was 0.003 but changed to 0.1 μM, as shown above in *Figure 17*. In contrast to 7-methoxycyclopropamitosene (57), this suggests that that a different enzyme is involved in its activation. In the case with 7-(aziridin-1-yl)cyclopropamitosene (157) substantial protection was observed, which implies that DT-diaphorase is important with regard to activation of this compound in air. It also suggests that 7-(aziridin-1-yl)cyclopropamitosene (157) might be useful in the treating cells types with high levels of DT-diaphorase. This compound (157) has potential and is also under going further investigation at this time.

From *Table 18*, a trend correlating increasing electron donating power of substituents at C-7 on ring A with less *in vitro* activity was observed. Comparing compounds (57) and (155), clearly the pyrrolidinyl group is a better donating group than the 7-methoxy of compound (57), and appears not as an effective drug under both aerobic and anaerobic conditions (140 μM under both conditions).

When compound, 7-(aziridin-1-yl)cyclopropamitosene (157) is compared with 7-(2-methylaziridin-1-yl)cyclopropamitosene (156) as shown in *Table 18*, a striking difference is observed. It is remarkable how such a small structural modification of a methyl group on the aziridine ring could dramatically alter both the IC_{50} values under aerobic and anaerobic conditions. Clearly, 7-(2-methylaziridin-1-yl)cyclopropamitosene (156) is not as an effective drug under both aerobic and anaerobic conditions. These reported results are similar to results by Workman *et al.* with EO9. Replacement of the aziridine group in EO9 with a 2-methylaziridine results in a 50 fold reduction in toxicity which is accompanied by substantial decrease in metabolism by DT-diaphorase. Whereas, replacement of the aziridine group with a methoxy group causes only a slight reduction in metabolism by DT-diaphorase, but toxicity is decreased a 100 fold. Studies are underway by Workman *et al.* to determine 7-methoxycyclopropamitosene (57), 7-(aziridin-1-yl)cyclopropamitosene (157), and 7-(2-methylaziridin-1-yl)cyclopropamitosene (156) are metabolised by DT-diaphorase.[60] This observation that a decrease in both the potency and antitumour effectiveness resulted when a 2-methyl group was added to 7-aziridine was also noted by Iyengar *et al.*[90] This same trend was observed when 2-aziridinyl compounds (160), (162), and (164) were compared to their analogous 2-methylaziridinyl derivatives (161), (163), an (165) as illustrated in *Table 18*.

Clearly, from *Table 18*, cyclopropamitosenes and mitosenes (160), (161), (78), (164), (165), and (133) without any leaving group at C-10 are not as potent under both aerobic and anaerobic conditions as the corresponding analogous compounds with the C-10 urethane groups (57), (157), (156), (162), (163), and (138). Cyclopropamitosene (78) without an alkylation site at C-10 is essentially inactive under aerobic and anaerobic conditions, as it has a IC_{50} value of 200 μM. These results suggest that a good leaving group at the C-10 position is necessary for antitumour activity, and this correlates well with the trends for formal reduction potential for the one electron reduction process in an non-aqueous solution (DMF).

In Summary: Some of these preliminary results appear to be very promising. 7-Methoxycyclopropamitosene (57) appears to be more specific for hypoxic cells than MMC, and at lower concentration under hypoxic conditions. 7-(Aziridin-1-yl)cyclopropamitosene (157) is equally effective at killing aerobic and hypoxic cells at extremely low conditions (0.003 μM). From the dicoumarol experiments, it is implied that DT-diaphorase is important with regard to the activation of this compound (157) in air. This suggests that 7-(aziridin-1-yl)cyclopropamitosene (157) might be useful in treating cell types with high levels of DT-diaphorase. There appears to be some correlation between the compounds potency and reduction potential;

(i) Cyclopropamitosene with inductive electron withdrawing substituents at the C-7 position are more potent *in vitro* under aerobic and anaerobic conditions, with the aziridinyl analogues (157), (160), (162), and (164) being the most active of a homologous series. Inductive electron withdrawing substituents at the C-7 position facilitate the one electron reduction process also.

(ii) Derivatives with the methyl group at the 2-position of aziridine were not as potent as the corresponding aziridine derivatives, which were also more difficult to reduce to the semiquinone.

(iii) Cyclopropamitosene analogues with good leaving groups at the C-10 position were more potent *in vitro* than the corresponding derivatives with no alkylation site at the C-10 position, which were also easier to reduce.

Hence, a correlation between these compounds potency and redox potential has been established. In the future, electrochemical measurements might be used as a screen for the biological activity of these compounds *in vitro*, but unfortunately it does not give any insight into the magnitude of the oxic / hypoxic differential. More information on the enzymatic activation of these compounds may be needed to allow prediction of these differentials.

5.2.3. *Further Biological Testing*

From preliminary results both compounds, 7-methoxycyclopropamitosene (57) and 7-(aziridin-1-yl)cyclopropamitosene (157) appear to be promising drugs and we decided to subject them to further investigation. Some of the other *in vitro* and *in vivo* results are now discussed. The activity of MMC, 7-methoxycyclopropamitosene (57), and 7-(aziridin-yl)cyclopropamitosene (157) in a range of cell lines V79, CHO-K1, AA8, and various mutants chosen for their differing repair capabilities, were recorded and summarised below in *Table 19*. The rationale for determining the activity of these drugs in a variety of cells lines is to enlighten biologists and chemists as to which enzymes are involved in the bioreductive activation process, and to help define the mechanism of action of these alkylation quinones.

Table 19 Toxicity of MMC, 7-Methoxycyclopropamitosene (57) and 7-(Aziridin-1-yl)cyclopropamitosene (157) in Various Rodent Cell Lines (used a colony forming assay)

Cell Line	Concentration (μM) to kill 90% cells following 3 hours in air		
	MMC	(57)	(157)
V79 (wild type)	1	7	0.02
IRS-1 (DNA strand break repair deficient: radiation sensitive)	0.02	1.5	<0.002
CHO-K1 (wild type)	1.5	0.6	0.2
CHO-MMC^R (low in NADPH : cytochrome P450 reductase)	25	3	0.8
AA8 (wild type)	1	2.0	0.06
UV41 (cross link repair deficient)	0.02	0.8	0.003
S162 (low in DT-diaphorase activity)	2.5	1.8	-
S932 (cross link repair deficient, and low in DT-diaphorase)	0.25	0.6	-

The spectrum of activity of MMC, 7-methoxycyclopropamitosene (57), and 7-(aziridin-1-yl)cyclopropamitosene (157) in a range of cells lines in shown above in *Table 19*.

Initially, MMC shall be discussed. IRS-1 and UV41 cell lines, which are deficient in their ability to repair DNA strand breaks and cross link repair mechanisms respectively are both hypersensitive to MMC (50 times). These results imply that MMC causes both DNA strands breaks and cross links with DNA. S932 cell line which is cross link repair deficient and low in DT-diaphorase (doublet mutant) is resistant to MMC (12.5 times) than the UV 41 cells from which they were derived. This result suggests that MMC is activated by DT-diaphorase even in cell lines deficient in cross link repair mechanism. MMC is effective killing CHO-MMC^R cells and the degree of resistance (MMC^R vs CHO-K1) of 16.7 is high, and probably indicates that MMC is also dependent on NADPH : cytochrome P450 reductase for activation. These results implies that MMC can probably be activated by both enzymes , DT-diaphorase or NADPH : cytochrome P450 reductase but not necessarily at the same time.

Now comparing MMC to 7-methoxycyclopropamitosene (57) in the same range of cells lines. 7-Methoxycyclopropamitosene (57) causes both DNA strand breaks and DNA cross links in IRS-1 and UV41 cell lines respectively but does not show a similar hypersensitivity as MMC. 7-Methoxycyclopropamitosene (57) is more potent in CHO-K1 and CHO-MMC^R, but is less effective in killing MMC^R cells (low on NADPH : cytochrome P450 reductase). The degree of resistance (MMC^R vs CHO-K1) is 5 and this suggests that compound (57) is in someway dependent on NADPH : cytochrome P450 reductase for activation. In contrast, cell lines such as S162 and S932 do not appear to be resistance to 7-methoxycyclopropamitosene (57), as both have ratios of approximately 1. This suggests that DT-diaphorase, probably plays no role in the activation of 7-methoxycyclopropamitosene (57) and confirms the obscurities with dicoumarol . These results suggest that 7-methoxycyclopropamitosene (57) causes both DNA strand breaks and DNA cross links, and NADPH : cytochrome P450 reductase is important for its activation.

Finally, comparing MMC to 7-(aziridin-1-yl)cyclopropamitosene (157). 7-(Aziridin-1-yl)cyclopropamitosene (157) shows parallel sensitivity to MMC in both the IRS-1 and UV41 cell lines, indicating that the nature of molecular damage caused by these drugs may be similar. Although 7-(aziridin-1-yl)cyclopropamitosene (157) is always more potent, this compound (157) is least effective in killing MMC^R cells but the degree of resistance (MMC^R vs CHO-K1) is much less than that observed for MMC and probably indicates that 7-(aziridin-1-yl)cyclopropamitosene (157) is less dependent on NADPH : cytochrome P450 reductase for activation.

Table 20: Toxicity of MMC, 7-Methoxycyclopropamitosene (57), and 7-(Aziridin-1-yl)cyclopropamitosene (157) towards murine bone marrow stem cells (CFU-A)

Drug	IC_{50} (μM)*
7-Methoxycyclopropamitosene (57)	3
7-(Azirdin-1-yl)cyclopropamitosene (157)	0.3
Mitomycin C	1

* Concentration required to reduce survival by 50% following exposure for 3 hours at 37°C in air.

The next set of experiments results are for mouse bone marrow stem cells exposed *in vitro* to either MMC, 7-methoxycyclopropamitosene (57) and 7-(aziridin-1-yl)cyclopropamitosene (157) as shown above in *Table 20*. Bone marrow toxicity is dose limiting for the use of MMC. There is only a 10 fold difference between 7-methoxycyclopropamitosene (57) and 7-(aziridin-1-yl)cyclopropamitosene (157) compared with a 1000 fold difference in V79 cells. This probably reflects differences in DT-diaphorase in the two different cell populations. *In vitro* the difference in toxicity between 7-(aziridin-1-yl)cyclopropamitosene (157) and MMC is only a factor of 3, which is far less than for other cell lines as shown in *Table 20*. This is a positive advantage in favour of 7-(aziridin-yl)cyclopropamitosene (157) for potential clinical use

Table 21: Effect of 7-(Aziridin-1-yl)cyclopropamitosene (157) on the Growth of RIF-1 Tumour

Treatment	Time to 4 x Volume / days
Untreated controls	5.0 ± 0.5
7-(Aziridin-1-yl) cyclopropamitosene (157)	7.1 ± 0.7
25 Gy X-Rays only	26.4 ± 2.8
25 Gy plus 7-(aziridin-1-yl) cyclopropamitosene (157)	32.2 ± 5.1
7-methoxycyclopropamitosene (57)	8.3 ± 0.9
25 Gy plus 7-methoxycyclopropamitosene (157)	35.3 ± 6.1

Finally, the *in vivo* results for 7-(aziridin-1-yl)cyclopropamitosene (157) are shown in *Table 21*. The effects of drugs *in vivo* are still more complex. The efficacies of some analogues as agents toxic to hypoxic cells appears to be related to the hypoxic / aerobic

differentials. However, other factors, such as distribution of the active drug into hypoxic tumour regions, and the nature of dose-limiting toxicities, clearly affect the antineoplastic efficacies and clinical potential of drugs. In these experiments Stratford *et al.* have used RIF-1 tumour and measured the effect of 7-(aziridin-1-yl)cyclopropamitosene (157) with and without radiation. Tumours implanted subcutaneosly on the backs of mice are treated when they reach a diameter of 5 x 5 x 5 mm; the end is time taken to reach 4 x the treatment volume. Each group consists of 6-8 mice. 7-(Aziridin-1-yl)cyclopropamitosene (157) gives a small anti-tumour effect when used alone at its maximum tolerated dose (30 days MTD = 4 mg / kg for MMC) and also provides a small increase in the effectiveness of radiation. However, this is small compared to MMC which inhibits growth for 40 days + when used with radiation. This lack of effectiveness of 7-(aziridin-1-yl)cyclopropamitosene (157) may be due to low metabolic stability and / or RIF-1 cells containing little DT-diaphorase. This requires further investigation.

In Summary: From preliminary results, the activation of 7-methoxycyclopropamitosene (57) and 7-(aziridin-1-yl)cyclopropamitosene (157) appears to be effected by different enzymes. However, the mechanism of activation for both these compounds can not be fully elucidated until further studies have been undertaken. The *in vitro* activity of 7-(aziridin-1-yl)cyclopropamitosene (157) appears promising, but the preliminary results *in vivo* are less encouraging, possibly because of low metabolic stability and / or due to RIF-1 cells containing little DT-diaphorase.

CHAPTER 6

EXPERIMENTAL PROCEDURES

6.1. General Information

Solvents and Reagents: Commercially available solvents and reagents were used throughout without further purification, except for those detailed below which were purified as described. 'Light petroleum' refers to the fraction of petroleum ether boiling between 40°C and 60°C and was distilled through a 36 cm Vigreux column before use. Diethyl ether and xylene were dried where necessary by standing over sodium wire for several days. THF was distilled from sodium benzophenone ketyl under nitrogen, prior to use. Dichloromethane and 1,2-dichloroethane were distillated from phosphorus pentoxide, prior to use. Ethyl acetate was stirred over anhydrous potassium carbonate for 15 h, decanted and distilled before storing over activated 4Å molecular sieves. Acetonitrile was dried over anhydrous potassium carbonate, and distilled from phosphorus pentoxide before storing over activated 4Å molecular sieves under nitrogen. DMF was dried by stirring over calcium hydride for 15 h, decanted, and distilled under reduced pressure before storing over activated 4Å molecular sieves under nitrogen. Pyridine, triethylamine and diisopropylamine were each distilled from, and stored over, potassium hydroxide pellets. Methanol and ethanol were distilled from magnesium turnings and iodine and stored over activated 4Å molecular sieves under nitrogen. Acetic anhydride was purified by fractional distillation through an efficient column and stored under nitrogen. Azirdine was prepared according to the literature procedure and 2-methylaziridine was commerically available. Both were distilled from potassium hydroxide pellets and stored over sodium hydroxide pellets under nitrogen in the refigerator. 2-Methoxyethanol was distilled from sodium and stored under nitrogen. 2-Methoxyethylamine and pyrrolidine were fractionally distilled, under nitrogen, and stored under nitrogen. Unless otherwise stated, all reagents were used as supplied.

Chromatographic Procedures: Analytical thin layer chromatography (TLC) was carried out using aluminium backed plates coated with Merck Kieselgel 60 GF_{254}. Plates were visualised under uv light (at 254 and / or 360 nm) or by staining with Ehrlich's reagent (a stain for NH bonds of indoles). Flash chromatography was carried out using Merck Kieselgel 60 H silica or Sorbsil C 60 silica gel. Pressure was applied at the column head with either hand bellows or an aquarium pump. Gravity chromatography was carried out using Merck Kieselgel 60 (70-230 mesh) silica. Samples were applied pre-adsorbed on silica or as a saturated solution in an appropriate solvent.

Spectroscopic techniques: Infra red spectra were recorded in the range 4000-600 cm^{-1} using a Pye-Unicam PU 9516 linked to an IBM computer or a Nicolet FT-205 spectrometer, both with internal calibration. Spectra were recorded as either solutions in

chloroform, as thin films or as nujol mulls. Thin films and nujol mulls were recorded between sodium chloride plates.

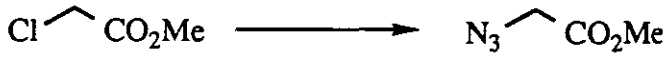
^1H NMR spectra were recorded using Bruker AC-250 (250 MHz), and Bruker WH-400 (400 MHz) (courtesy of the SERC NMR Spectroscopy Centre, Warwick) instruments. ^{13}C NMR spectra were recorded on a Bruker AC-250 (62.9 MHz), Bruker WH-400 (100.6 MHz). ^1H NMR spectra are referenced against tetramethylsilane at 0.0 ppm when using the continuous wave machine and against residual undeuterated solvent when using the Fourier transform machines. In the case of deuterochloroform, this is 7.260 ppm. Signals are described as singlets (s), doublets (d), triplets (t), quintets (q), multiplets (m), double doublets (dd), two overlapping doublets (2d) etc.

High and low resolution mass spectra were recorded on a Kratos MS80 instrument or on a VG Analytical ZAB-E instrument (courtesy of the SERC mass spectometry service, Swansea).

Other data and instrumentation: Melting points were measured on a Reichert-Kofler hot stage apparatus or an Electrothermal digital melting point apparatus and are uncorrected.

Note: Synthesis or preparation of general reagents

Methyl Azidoacetate



Methyl chloroacetate (50.0 g, 0.461 mol) was added followed by sodium azide (37.65 g, 0.579 mol) to a stirred mixture of water (50 cm^3) and acetone (75 cm^3). The stirred mixtre was refluxed for 16 h. After this time, the mixture was cooled to room temperature, and the acetone removed in vacuo to afford a yellow liquid / white solid (sodium chloride). The mixture of liquid and solid was then extracted with ether (3 x 150 cm^3). The ethereal extracts were washed with water (3 x 100 cm^3), brine (150 cm^3), dried ($MgSO_4$), and then condensed in vacuo to give the azide (44.2 g, 83%) as a pale yellow liquid, δ_H (270 MHz, $CDCl_3$+TMS) 3.90 (2 H, s, CH_2), and 3.81 (3 H, s, Me). Note: Care was taken at all times when handling this compound. Methyl azidoacetate is is potentially explosive. Hence, this compound was not purified by distillation.

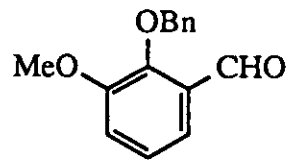
Manganese Dioxide

A solution of manganese (II) sulphate tetrahydrate (223 g, 1 mol) in water (300 cm^3), and a solution 40% sodium hydroxide (240 cm^3, 2.5 mol) were added simultaneously over a period of one hour to a stirred hot solution of potassium permanganate (190 g, 1.2 mol) in water (1200 cm^3). After one hour of stirring at the same temperture, the mixture was filtered and a brown precipitate was isolated. The brown solid was washed thoroughly with water until the washings went colourless. The product was dried overnight and the remaining water was removed by azeotropic distillation with benzene (150 cm^3). The solvent was removed in vacuo yielding a fine brown solid, which was kept in the oven at 100°C at all times so that the manganese dioxide was active prior to use.

Fremy's Salt $[K_2ON\ (SO_3)_2]$

A solution of sodium nitrite (5.8 g, 0.08 mol) in water (15 cm^3) was well cooled in an ice bath, and crushed ice (35 g) stirred in, with continuous stirring. A solution of sodium metabisulphite (7.3 g, 0.04 mol) in water (15 cm^3) was added next, followed by glacial acetic acid (3.5 cm^3). The mixture was rendered alkaline by adding ammonia (0.88, 2.5 cm^3), and was then stirred continuously in the ice bath during addition of an ice cold solution of potassium permanganate (2.1 g, 0.013 mol) in water (65 cm^3). The precitate of manganese dioxide was filtered off through a bed a Celite. To the cooled violet filtrate was added a saturated solution (85 cm^3) of potassium chloride (33 g / 100 cm^3 of water). An orange solid precipitated out, which was then filtered under suction. The orange solid was washed several times with 5% potassium hydroxide, then twice with ethanol containing approximately 5% v / v 0.88 ammonia and finally with acetone. Air was not drawn through the solid but it was spread on a watch glass and the acetone allowed to evaporate over 10 - 15 min. The product was dried in a desiccator over ammonium carbonate and calcium oxide.

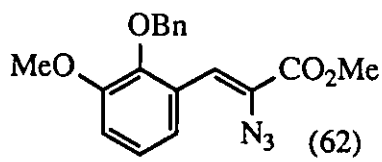
6.2. Experimental For 2.2.



(61)

2-Benzyloxy-3-methoxybenzaldehyde (61)

Potassium hydroxide pellets (40.6 g, 724 mmol) were added to a stirred solution of *o*-vanillin (60) (100.0 g, 657 mmol) in ethanol (99.7%, 590 cm^3), followed by benzyl chloride (83.45 cm^3, 725 mmol). The stirred mixture was refluxed for 12 h, then water (100 cm^3) was added, and the mixture extracted with diethyl ether (3 x 750 cm^3). The ethereal extracts were washed with water (3 x 200 cm^3), potassium hydroxide solution (2 M, 5 x 500 cm^3), again with water (2 x 150 cm^3), then brine (150 cm^3). The organic layer was dried ($MgSO_4$), then condensed in vacuo, to give the title compound (61) (99.2 g, 62%) as a colourless solid on trituration with light petroleum, m.p. 45-47°C (lit.[79] 41-43°C), $\nu_{max.}$ (Nujol) 1697, 1584, 1367, 1265, 1247, and 1221 cm^{-1}; δ_H (270 MHz; $CDCl_3$) 10.24 (1 H, s, CHO), 7. 41-7.14 (8 H, m, Ar-H), 5.18 (2 H, s, OCH_2Ph), and 3.95 (3 H, s, OMe); δ_C (62.9 MHz; $CDCl_3$+TMS) 190.16 (CH, CHO), 153.04 (C), 151.02 (C), 136.39 (C), 130.27 (C), 128.66 (CH), 128.57 (CH), 128.49 (CH), 124.24 (CH), 118.96 (CH), 117.99 (CH), 76.27 (CH_2, OCH_2Ph), and 56.04 (CH_3, OMe); m/z 243 (MH^+, 11%), 242 (M^+, 62), 213 (27), and 91 (100).

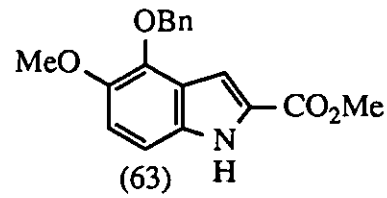


(62)

Methyl 2-Azido-3-(2-benzyloxy-3-methoxyphenyl)propenoate (62)

Sodium metal (12.54 g, 545 mmol) was added to dry methanol (300 cm^3). The solution was cooled to -15°C, and a solution of methyl azidoacetate (61.00 g, 530 mmol) and 2-benzyloxy-3-methoxybenzaldehyde (61) (33.07 g, 137 mmol) in dry methanol (25 cm^3) was added dropwise by syringe. The mixture was stirred at -10°C for 3 h then at 4°C for 12 h. Water (100 cm^3) was cautiously added to the mixture, which was then extracted

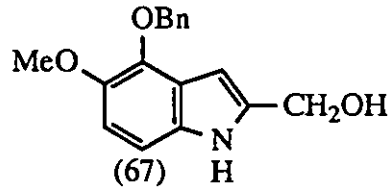
with ethyl acetate (3 x 500 cm^3). The combined extracts were washed with water (3 x 500 cm^3), brine (300 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a pale yellow residue, which was triturated with a small quantity of diethyl ether and the resulting precipitate was filtered off. The remaining oily residue was purified by column chromatography (eluting with 50% light petroleum : 50% diethyl ether) to give title compound (62) (38.03 g, 82%) as pale yellow crystals, $\nu_{max.}$ (film) 2121, 1712, 1456, 1378, 1260, and 1218 cm^{-1}; δ_H (270 MHz; $CDCl_3$) 7.77 (1 H, dd, J 1.4, 8.0 Hz Ar 6-H), 7.44-7.27 (5 H, m, Bn-H), 7.26 (1 H, s, CH=), 7.11 (1 H, t, J 8.0 Hz, 5-H), 6.95 (1 H, dd, J 1.5, 8.0 Hz, 4-H), 5.00 (2 H, s, OCH_2Ph), 3.90 (3 H, s, CO_2Me), and 3.85 (3 H, s, OMe).



(63)

Methyl 4-Benzyloxy-5-methoxyindole-2-carboxylate (63)

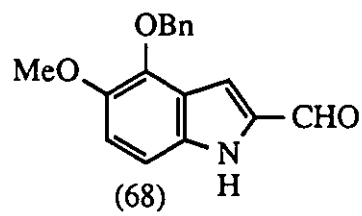
A solution of methyl 2-azido-3-(2-benzyloxy-3-methoxy phenyl)propenoate (62) (14.6 g, 43 mmol), in dry xylene (370 cm^3) was introduced, dropwise, by means of a pressure equalising dropping funnel, to refluxing dry xylene (1250 cm^3). After the addition was complete (ca. 30 min), the solution was refluxed for a further 45 min. Removal of solvent in vacuo gave a brown residue. The residue was triturated with a small quantity of diethyl ether and the resulting precipitate was filtered off. The remaining oily residue was purified by column chromatography (eluting with 50% light petroleum : 50% diethyl ether) to give the title compound (63) (8.8 g, 66%) as a pale yellow solid, m.p. 114-116°C (lit.[79] 110-111°C), $\nu_{max.}$ ($CHCl_3$) 3462, 3013, 1707, 1533, 1453, and 1253 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 9.36 (1 H, s, NH), 7.54 (2 H, d, J 7.1 Hz, Bn-H), 7.53-7.29 (4 H, m, 3 Bn-H and 3-H), 7.09 (2 H, s, 6-H and 7-H), 5.29 (2 H, s, OCH_2Ph), 3.94 (3 H, s, MeO/CO_2Me), and 3.89 (3 H, s, CO_2Me/OMe); δ_C (62.9 MHz; $CDCl_3$+TMS) 162.46 (CO, CO_2Me), 145.17 (C), 141.78 (C), 137.91 (C), 134.14 (CH), 128.56 (CH), 128.36 (CH), 128.04 (CH), 127.34 (C), 123.18 (C), 116.33 (CH), 107.14 (CH), 106.17 (CH), 75.06 (CH_2, OCH_2Ph), 58.49 (CH_3, OMe), and 52.02 (CH_3, CO_2Me).



(67)

4-Benzyloxy-5-methoxyindole-2-methanol (67)

A solution of methyl 4-benzyloxy-5-methoxyindole-2-carboxylate (63) (6.975 g, 22.4 mmol) in dry THF (200 cm^3) was added dropwise to a stirred suspension of lithium aluminium hydride (1.700 g, 44.8 mmol) in dry THF (85 cm^3), such that the mixture achieved gentle reflux. After 30 min, water (1.7 cm^3), 15% sodium hydroxide (1.7 cm^3), and water again (5.1 cm^3), were added to the mixture, and the resultant precipitate removed by filtration (through a bed of Celite). The filtrate was dried ($MgSO_4$), then condensed in vacuo. The residue was recrystallised from dichloromethane-light petroleum to give the title compound (67) (5.816 g, 92%) as a colourless crystalline solid, m.p. 90-91°C (lit.[79] 83-85°C), $\nu_{max.}$(Nujol) 3476, 3284, 1504, 1324, 1284, 1244, 1090, 1016, and 702 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 8.25 (1 H, br s, NH), 7.52 (2 H, m, Bn-H), 7.41-7.30 (3 H, m, Bn-H), 7.00 (1 H, d J 8.4 Hz, 7/6-H), 6.91 (1 H, d, J 8.6 Hz, 6/7-H) 6.40 (1 H, s, 3-H), 5.20 (2 H, s, CH_2OPh), 4.75 (2 H, s, CH_2OH), and 3.88 (3 H, s, OMe).

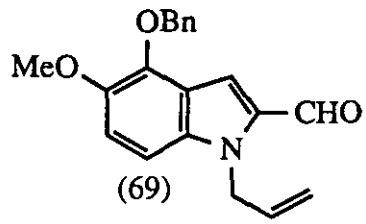


(68)

4-Benzyloxy-5-methoxyindole-2-carboxaldehyde (68)

Manganese dioxide (17.39 g, 200.00 mmol) was added, in portions to a stirred solution of 4-benzyloxy-5-methoxy-4-methylindole-2-methanol (67) (5.82 g, 20.56 mmol) in dry dichloromethane (650 cm^3). The suspension was then refluxed for 12 h. The mixture was filtered through a bed of Celite, and the residue washed with hot dichloromethane (3 x 300 cm^3). The combined filtrate and washings were evaporated to give an oil which was purified by column chromatography (eluting with dichloromethane) to give the title compound (68) (3.56 g, 62%) as a yellow crystalline solid, m.p. 143-145°C (lit.[79] 130-131°C), $\nu_{max.}$ (Nujol) 3458, 3015, 2837, 1663, 1531, and 1143 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 9.76 (1 H, s, CHO), 9.25 (1 H, s, NH), 7.49 (2 H, m, Bn-H), 7.39-7.30 (3 H, m, Bn-H), 7.24 (1 H, dd, J 0.8, 2.2 Hz, 3-H), 7.17 (1 H, d, J 8.9 Hz, 7/6-H), 7.12 (1

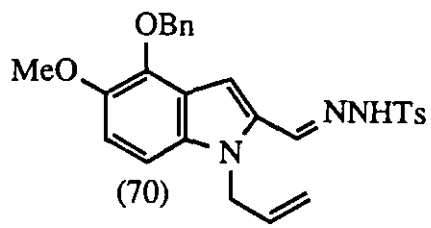
H, dd, J 0.8, 8.9 Hz, 6/7-H), 5.28 (2 H, s, CH_2OPh), and 3.91 (3 H, s, OMe); δ_C (62.9 MHz; $CDCl_3$+TMS) 182.14 (CH, CHO), 145.23 (C), 142.23 (C), 137.81 (C), 136.23 (C), 135.22 (C), 128.42 (CH), 128.14 (CH), 128.03 (CH), 123.43 (C), 118.41 (CH), 112.55 (CH), 107.67 (CH), 75.22 (CH_2, OCH_2Ph), and 58.46 (CH_3, OMe).



(69)

1-Allyl-4-benzyloxy-5-methoxyindole-2-carboxaldehyde (69)

To a flask charged with sodium hydride (80%, 0.475 g, 15.84 mmol) was added dry light petroleum (10 cm^3). The mixture was stirred for 10 min, the petroleum removed by syringe, and the flask contents dried under vacuum. 4-Benzyloxy-5-methoxyindole-2-carboxaldehyde (68) (3.562 g, 12.68 mmol) in DMF (260 cm^3) was added dropwise, and the mixture was stirred at room temperature for 30 min. Allyl bromide (1.36 cm^3, 15.76 mmol) was added, and the mixture was stirred at room temperature. After 1 h, water (150 cm^3) was cautiously added, and the mixture was extracted with ethyl acetate (4 x 250 cm^3). The combined extracts were washed with water (8 x 200 cm^3), brine (200 cm^3), dried ($MgSO_4$), and evaporated to give the title compound (69) (4.031 g, 99%) as a brown solid, $\nu_{max.}$ (Nujol) 1670, 1490, 1406, 1250, and 1140 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 9.79 (1 H, s, CHO), 7.51 (2 H, m, Bn-H), 7.38 (3 H, m, Bn-H), 7.25 (1 H, s, 3-H), 7.19 (1 H, d, J 9.0 Hz, 7/6-H), 7.04 (1 H, d, J 9.0 Hz, 6/7-H), 5.96 (1 H, m, $CH_2CH=CH_2$), 5.28 (2 H, s, CH_2OPh), 5.13 (3 H, m, $CH_2CH=CH_2$ and $CH_2CH=CHH$-cis), 4.89 (1 H, dd, J 1.0, 17.1 Hz, $CH_2CH=CHH$-trans), and 3.92 (3 H, s, OMe).

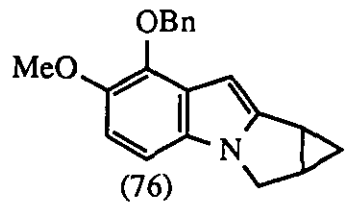


(70)

1-Allyl-4-benzyloxy-5-methoxyindole-2-carboxaldehyde tosylhydrazone (70)

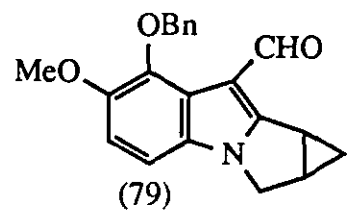
1-Allyl-4-benzyloxy-5-methoxyindole-2-carboxaldehyde (69) (3.095 g, 9.642 mmol) was added to a stirred solution of 4-toluenesulphonyl hydrazide (2.335 g, 12.539 mmol) in methanol (25 cm^3). After stirring at 40°C for 1 h, the solvent was removed in vacuo,

and the residue purified by column chromatography (eluting with diethyl ether), to give the title compound (70) (4.694 g, 99%) as a pale yellow solid, $\nu_{max.}$ ($CHCl_3$) 2956, 1718, 1492, 1456, 1358, and 1166 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 7.83 (2 H, m, Ar-H [AA'BB']), 7.73 (2 H, m, NH and C<u>H</u>=N), 7.47 (2 H, m, Bn-H), 7.36-7.28 (5 H, m, 3 Bn-H and 2 Ar-H [AA'BB']), 7.03 (1 H, d, J 8.8 Hz, 6/7-H), 6.94 (1 H, d, J 8.8 Hz, 7/6-H), 6.67 (1 H, s, 3-H), 5.85 (1 H, m,-C<u>H</u>=CH_2), 5.20 (2 H, s, OCH_2Ph), 5.04 (3 H, m, $C\underline{H}_2CH=CH_2$ and $CH_2CH=C\underline{H}H$-cis), 4.85 (1 H, dd, J 1.2, 17.2 Hz, $CH_2CH=CH\underline{H}$-trans), 3.88 (3 H, s, OMe), and 2.41 (3 H, s, Ar-Me).



(76)

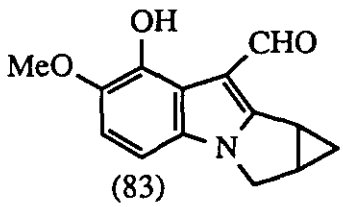
8-<u>Benzyloxy</u>-7-<u>methoxy</u>-1,2-<u>dihydro</u>-3H-1,2-<u>cyclopropapyrrolo</u>[1,2-a]<u>indole</u> (76)
Sodium hydride (80%, 0.237 g, 7.907 mmol) was added to a stirred solution of the tosylhydrazone (70) (2.602 g, 5.321 mmol) in dry THF (80 cm^3). After 25 min, the solution was filtered, and the filtrate evaporated. The residue was dissolved in dry chlorobenzene (800 cm^3), and the solution refluxed for 3 h. The solvent was evaporated, and the residue purified by chromatography to give the title compound (70) (1.530 g, 94%) as a colourless oil, $\nu_{max.}$ (film) 1560, 1492, 1258, 1232, and 744 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 7.55 (2 H, m, Bn-H), 7.42-7.29 (3 H, m, Bn-H), 6.81 (2 H, 2d, J 8.6 Hz, 5-H and 6-H), 6.22 (1 H, s, 9-H), 5.20 (2 H, s, OCH_2Ph), 4.07 (2 H, m, 3-CH_2), 3.89 (3 H, s, OMe), 2.37 (2 H, m, 1-H and 2-H), 1.25 (1 H, m, 1a-C<u>H</u>H), and 0.64 (1 H, m, 1a-CH<u>H</u>).



(79)

8-<u>Benzyloxy</u>-7-<u>methoxy</u>-1,2-<u>dihydro</u>-3H-1,2-<u>cyclopropapyrrolo</u>[1,2-a]<u>indole</u>-9-<u>carboxaldehyde</u> (79)
N-Methylformanilide (1.222 g, 9.037 mmol) and phosphorus oxychloride (1.386 g, 9.037 mmol) were stirred under a calcium oxide drying tube for 20 min. The resulting precipitate was cooled to 0°C, and 1,2-dichloroethane (34 cm^3) was added. 8-Benzyloxy-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole (76) (2.297

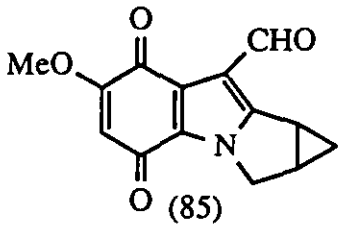
g, 7.531 mmol) was added, and the mixture was refluxed for 1.25 h. Sodium acetate (1 M, 49 cm^3) was added, and the mixture was extracted with ethyl acetate (2 x 200 cm^3). The combined extracts were washed with water (3 x 250 cm^3), brine (200 cm^3), dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography gave the title compound (79) (1.902 g, 76%) as a colourless solid, $\nu_{max.}$ (Nujol) 1638, 1604, 1522, and 742, cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.32 (1 H, s, CHO), 7.49 (2 H, m, Bn-H), 7.41-7.26 (3 H, m, Bn-H), 6.88 (2 H, 2d, J 8.6 Hz, 5-H and 6-H), 5.16 (2 H, s, OCH_2Ph), 4.13 (2 H, m, 3-CH_2), 3.92 (3 H, s, OMe), 3.00 (1 H, m, 1-H), 2.48 (1 H, m, 2-H), 1.49 (1 H, m, 1a-C<u>H</u>H), and 0.72 (1 H, m, 1a-CH<u>H</u>).



(83)

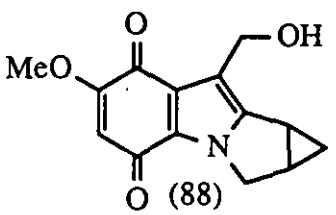
8-Hydroxy-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde (83)

To a solution of 8-benzyloxy-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde (79) (0.500 g, 1.501 mmol) in ethyl acetate (250 cm^3) was added 10% palladium on carbon catalyst (0.100 g), and dilute sulphuric acid (8 drops). The mixture was stirred under an atmosphere of hydrogen for 2 h. After this time, the suspension was filtered and washed with dichloromethane. The filtrate and washings were extracted with water (3 x 100 cm^3), brine (75 cm^3), and dried ($MgSO_4$). The organic layer was evaporated to dryness to give a brown solid residue. Purification of the residue by column chromatography gave title compound (83) (0.288 g, 79%) as a colourless solid, $\nu_{max.}$ (Nujol) 1606, 1304, 1252, and 824 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.87 (1 H, s, CHO), 9.58 (1 H, s, OH), 6.88 (1 H, d, J 8.5 Hz, 6/5-H), 6.52 (1 H, d, J 8.5 Hz, 5/6-H), 4.13 (2 H, m, 3-CH_2), 3.91 (3 H, s, OMe), 2.72 (1 H, m, 1-H), 2.57 (1 H, m, 2-H), 1.51 (1 H, m, 1a-C<u>H</u>H), and 0.81 (1 H, m, 1a-CH<u>H</u>).



(85)

9-Formyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione. (85) Potassium nitrosodisulphonate (1.405 g, 5.243 mmol) in water (70 cm^3), was added to a stirred solution of 8-hydroxy-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde (83) (0.579 g, 2.383 mmol) in acetone (232 cm^3). The mixture was buffered with sodium dihydrogen phosphate solution (0.167 M, 70 cm^3). After stirring at room temperature for 12 h, the mixture was concentrated in vacuo, filtered, and the residue recrystallised from dichloromethane-light petroleum to give the title compound (85) (0.581 g, 95%) as orange crystals, $\nu_{max.}$ (Nujol) 1680, 1662, 1638, 1586, 1500, 1244, and 1212 cm^{-1}; δ_H (250 MHz; $CDCl_3$ +TMS) 10.37 (1 H, s, CHO), 5.68 (1 H, s, 6-H), 4.32 (2 H, m, 3-CH_2), 3.85 (3 H, s, OMe), 2.86 (1 H, m, 1-H), 2.47 (1 H, m, 2-H), 1.47 (1 H, m, 1a-CHH), and 0.65 (1 H, m, 1a-CHH).

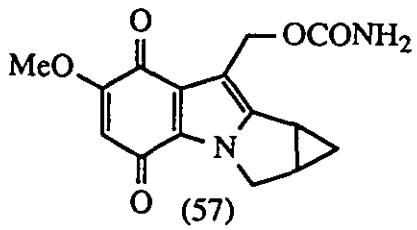


(88)

9-Hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5-8-dione (88)

Sodium borohydride (0.189 g, 4.996 mmol) was added to a stirred solution of 9-formyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (85) (0.176 g, 0.685 mmol) in methanol (132 cm^3) and the solution rigorously degassed with nitrogen. After stirring for 4 h at room temperature, air was blown through the solution rapidly, and the mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 200 cm^3). The combined extracts were washed with water (2 x 200 cm^3), brine (300 cm^3), and dried ($MgSO_4$). The solvent was evaporated, and the residue purified by column chromatography to give the title compound (88) (0.159 g, 90%) as a red solid, $\nu_{max.}$ (Nujol) 3312, 1668, 1630, 1586, 1586, and 722 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.61 (1 H, s, 6-H), 4.68 (2 H, m, 10-CH_2), 4.26 (2 H, m, 3-

CH_2), 3.89 (1 H, t, J 7.1 Hz, OH), 3.82 (3 H, s, OMe), 2.36 (2 H, m, 1-H and 2-H), 1.30 (1 H, m, 1a-CHH), and 0.60 (1 H, m, 1a-CHH).

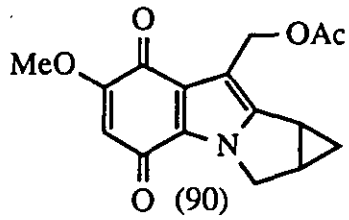


(57)

9-Hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57)

Phenyl chloroformate (0.08 cm^3, 0.618 mmol) was added dropwise to a stirred, ice cold solution of the alcohol (88) (0.100 g, 0.386 mmol) in dry pyridine (25 cm^3). The mixture was stirred at room temperature for 2 h, then water (10 cm^3) was added. The mixture was extracted with dichloromethane (3 x 50 cm^3), and the combined extracts were washed with water (3 x 50 cm^3), brine (100 cm^3), and dried ($MgSO_4$). The solvent was evaporated, and the residue purified by column chromatography to give an orange gummy solid.

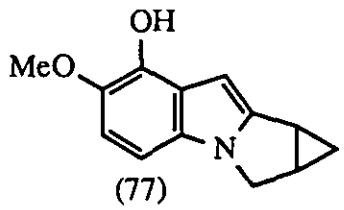
A solution of phenyl carbonate (89) in dry dichloromethane (55 cm^3),was cooled to -78°C. Ammonia gas was bubbled into the solution for approximately 45 min (monitored the reaction by TLC), after which time the contents were allowed to warm to room temperature, and the solvent removed in vacuo. Recrystallisation of the residue from dichloromethane-light petroleum gave the title compound (57) (0.097 g, 83%) as a red crystalline solid, m.p. 176-178°C (lit.[79] 175-177°C), $\nu_{max.}$ (Nujol) 3408, 3212, 1764, 1668 ,1620, 1584, 1350, 1306, and 1242 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.59 (1 H, s, 6-H), 5.29 (2 H, m, 10-CH_2),4.60 (2 H, br s, NH_2), 4.27 (2 H, m, 3-CH_2), 3.80 (3 H, s, OMe), 2.54 (1 H, m, 1-H), 2.33 (1 H, m, 2-H), 1.31 (1 H, m, 1a-CHH), and 0.58 (1 H, m, 1a-CHH); δ_C (100.6 MHz; $CDCl_3$) 177.68 (CO, 8-C), 177.20 (CO, 5-C), 160.44 (C/CO, 7-C/urethane), 156.77 (CO/C, 7-C/urethane), 146.31 (C, 4a-C), 129.24 (C, 8a/9a-C), 123.90 (C, 9a/8a-C), 111.33 (C, 9-C), 105.35 (CH, 6-C), 57.87 (CH_2, 10-C), 56.38 (CH_3, OMe), 50.00 (CH_2, 3-C), 20.63 (CH, 1-C), 16.17 (CH_2, 1a-C), 14.69 (CH, 2-C).



(90)

9-Acetoxy-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (90)

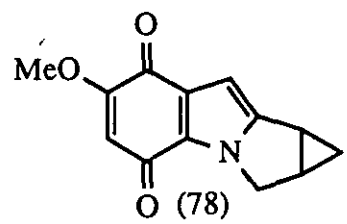
To a solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo-[1,2–a]indole-5,8-dione (88) (0.080 g, 0.309 mmol), in distilled pyridine (8.0 cm^3), was added acetic anhydride (1.5 cm^3), which was stirred at room temperture under nitrogen for 15 h. After this time, water (2 cm^3) was added. The mixture was extracted with dichloromethane (3 x 25 cm^3), and the combined extracts were washed with water (3 x 20 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography (eluting with ethyl acetate), to give an orange solid. Recrystallisation of this solid from dichloromethane-light petroleum gave the title compound (90) (0.089 g, 96%), as an orange solid, m.p. 164-166°C, (Found: M^+, 301.0954. $C_{16}H_{15}NO_5$ requires M, 301.0950); $\lambda_{max.}$ (MeOH) 236 (log ε 4.29), 291 (4.23), 348 (3.53), and 467 nm (3.17); $\nu_{max.}$ ($CHCl_3$) 2956, 2924, 1730, 1672, 1636, 1594, and 1230 cm^{-1}; δ_H (250 MHz, $CDCl_3$+TMS) 5.59 (1 H, s, 6-H), 5.28 (2 H, m, 10-CH_2), 4.27 (2 H, m, 3-CH_2), 3.80 (3 H, s, OMe), 2.45 (1 H, m, 1-H), 2.35 (1 H, m, 2-H), 2.08 (3 H, s, Me), 1.31 (1 H, m, 1a-C<u>H</u>H), and 0.57 (1 H, m ,1a-CH<u>H</u>); δ_C (100.6 MHz; $CDCl_3$) 177.76 (CO, 8-C), 177.20 (CO, 5-C), 170.87 (CO, ester), 160.52 (C, 7-C), 146.17 (C, 4a-C),126.52 (C, 9a/8a-C), 124.04 (C, 8a/9a-C), 110.73 (C, 9-C), 105.36 (CH, 6-C), 57.49 (CH_2, 10-C), 56.40 (CH_3, OMe), 50.00 (CH_2, 3-C), 20.88 (CH/CH_3, 1-C/Me), 20.67 (CH/CH_3, 1-C/Me), 16.14 (CH_2, 1a-C), and 14.61 (CH, 2-C); m/z 301 (M^+, 10%), 258 (100), 242 (39), 226 (6), 212 (12), 198 (12), 43 (37), and 28 (13).



(77)

7-Methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indol-8-ol (77)

To a solution of 8-benzyloxy-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole (76) (0.500 g, 1.656 mmol) in distilled ethyl acetate (250 cm^3) was added 10% palladium on carbon (0.100 g), and dilute sulphuric acid (5 drops). The mixture was stirred under an atmosphere of hydrogen for 5 h . After this time, the suspension was filtered and washed with dichloromethane (3 x 75 cm^3). The filtrate and washings were extracted with water (3 x 50 cm^3), brine (50 cm^3), and dried ($MgSO_4$). The organic layer was condensed in vacuo to give an oil. Purification of the residue by column chromatography (gradient elution with 100% light petroleum - 20% ethyl acetate : 80% light petroleum) gave the title compound as a colourless oil (77) (0.179 g, 50%), (Found: M^+, 215.0942. $C_{13}H_{13}NO_2$ requires M, 215.0946); $\nu_{max.}$ ($CHCl_3$) 3537 (sharp), 1510, 1458, 1255, and 1241 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.78 (1 H, d, J 8.6 Hz, 5/6-H), 6.59 (1 H, d, J 8.6 Hz, 6/5-H), 6.23 (1H, s, 9-H), 5.83 (1 H, s, OH), 4.04 (2 H, m, 3-CH_2), 3.87 (3 H, s, OMe), 2.45-2.27 (2 H, m, 1-H and 2-H), 1.24 (1 H, m, 1a-CHH), and 0.62 (1 H, m, 1a-CHH), δ_C (62.9 MHz; $CDCl_3$+TMS) 146.66 (C, 8-C), 138.83 (C, 7-C), 138.02 (C), 130.48 (C), 121.97 (C), 107.78 (CH, 5/6-C), 100.05 (CH, 6/5-C), 88.71 (CH, 9-C), 58.39 (CH_3, OMe), 46.58 (CH_2, 3-C), 21.44 (CH, 1-C), 17.22 (CH_2, 1a-C), and 15.82 (CH, 2-C); m/z 216 (MH^+, 18%), 215 (M^+, 96), 200 (100), and 172 (23).



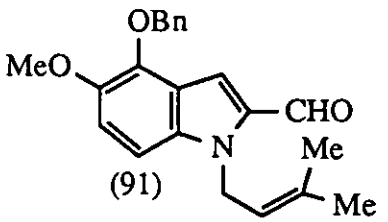
(78)

7-Methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (78)

Potassium nitrosodisulphonate (Fremy's salt) (0.491 g, 1.832 mmol) in water (24 cm^3) was added to a stirred solution of 7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indol-8-ol (77) (0.179 g, 0.833 mmol) in acetone (81 cm^3). The mixture was buffered with sodium dihydrogen phosphate solution (0.167 M, 24 cm^3). After stirring at room temperature for 15 h, the acetone was removed in vacuo.

The aqueous layer was then extracted with dichloromethane (3 x 75 cm^3). The organic extracts were washed with water (3 x 75 cm^3), brine (100 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography (eluting with diethyl ether) to give an orange solid. Recrystallisation of this orange residue from dichloromethane-light petroleum gave the title compound (78) (0.125 g, 65%) as an orange solid, m.p. 185-186°C, (Found: $\underline{M}^+$, 229.074. $C_{13}H_{11}NO_3$ requires M, 229.074); $\lambda_{max.}$ (MeOH) 235 (log ε 4.44), 289 (4.40), 345 (3.65), and 463 nm (3.26); $\nu_{max.}$ ($CHCl_3$) 1676, 1641, 1596, 1499, 1476, and 1237 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.30 (1 H, s, 9-H), 5.57 (1 H, s, 6-H), 4.27 (2 H, m 3-CH_2), 3.60 (3 H, s, OMe), 2.36 (2 H, m, 1-H and 2-H), 1.29 (1 H, m 1a-C$\underline{H}$H), and 0.59 (1 H, m, 1a-CH$\underline{H}$); δ_C (62.90 MHz; $CDCl_3$+TMS) 177.77 (CO, 8-C), 177.09 (CO, 5-C), 160.70 (C, 7-C), 146.75 (C, 4a-C), 127.39 (C, 9a/8a-C), 126.70 (C, 8a/9a-C), 105.65 (CH, 6-C), 99.19 (CH, 9-C), 56.50 (CH_3, OMe), 49.92 (CH_2, 3-C), 21.05 (CH, 1-C), 16.36 (CH_2, 1a-C), and 15.23 (CH, 2-C); m/z 230 ($\underline{MH}^+$, 15%), 229 ($\underline{M}^+$, 100), 200 (34), 186 (33), and 51 (33).

6.3. Experimental For 2.3.

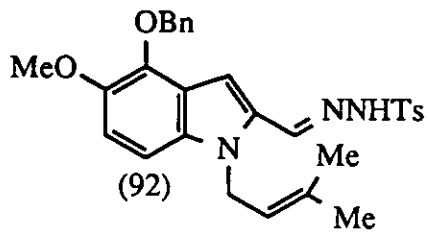


(91)

4-Benzyloxy-5-methoxy-1-(2-methyl-2-butenyl)indole-2-carboxaldehyde (91)

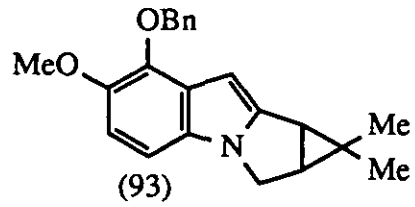
To a flask charged with sodium hydride (80%, 0.300 g, 10.000 mmol) was added dry light petroleum (5 cm^3). The mixture was stirred for 10 min, the petroleum removed by syringe, and the flask contents dried under vacuum. 4-Benzyloxy-5-methoxyindole-2-carboxaldehyde (68) (2.340 g, 8.327 mmol) in DMF (170 cm^3) was added dropwise, and the mixture was stirred at room temperature for 45 min. 4-Bromo-2-methylbutene (1.15 cm^3, 10.000 mmol) was added, and the mixture was stirred at room temperature. After 15 h, water (20 cm^3) was cautiously added, and the mixture was extracted with ethyl acetate (3 x 150 cm^3). The combined extracts were washed with water (8 x 150 cm^3), brine (150 cm^3), dried ($MgSO_4$), and evaporated to dryness. The residue was purified by column chromatography (eluting with 50% diethyl ether : 50% light petroleum) to give the title compound (91) (1.894 g, 65%) as a pale yellow solid, m.p.

55-57°C, (Found: C, 75.4; H, 6.6; N, 3.9. $C_{22}H_{23}NO_3$ requires C, 75.6; H, 6.6; N, 4.0%); $\nu_{max.}$ ($CHCl_3$) 1666, 1516, 1488, 1460, 1290, 1248, and 1140 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 9.79 (1 H, s, CHO), 7.51-7.00 (8 H, m, 5 Ar-H and 3, 6, 7-H), 5.27 (2 H, s, OCH_2Ph), 5.18 (3 H, m, $NCH_2CH=CMe_2$ and $CH_2CH=CMe_2$), 3.91 (3 H, s, OMe), 1.86 (3 H, s, Me), and 1.68 (3 H, s, Me); m/z 350 (MH^+, 7%), 349 (M^+, 28), 258 (41), 190 (100), 91 (60), 69 (93), and 41 (71).



(92)

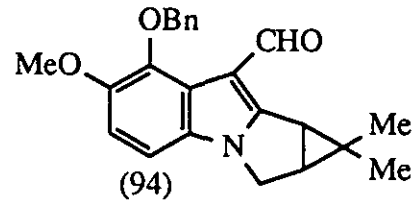
4-Benzyloxy-5-methoxy-1-(2-methyl-2-butenyl)indole-2-carboxaldehyde tosylhydrazone. (92) 4-Toluenesulphonyl hydrazide (1.516 g, 8.141 mmol) in dry methanol (20 cm^3) was added to a stirred solution of 4-benzyloxy-5-methoxy-1-(2-methyl-2-butenyl)indole-2-carboxaldehyde (91) (1.894 g, 5.427 mmol) in dry methanol (30 cm^3). After stirring at 40°C for 15 h, the solvent was removed in vacuo, and the residue was purified by chromatography (70% diethyl ether : 30% light petroleum) to give a cream foam. Recrystallisation of this foam from dichloromethane-light petroleum gave the title compound (92) (1.681 g, 60%) as a colourless solid, m.p. 77-79°C, (Found: C, 67.0; H, 6.0; N, 8.2. $C_{29}H_{31}N_3O_4S$ requires C, 67.3; H, 6.0; N, 8.1%); $\nu_{max.}$ ($CHCl_3$) 3184, 1608, 1598, 1516, 1490, 1454, 1344, and 1166 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 7.82 (2 H, m, Ar-H [Ts-AA'BB']), 7.74 (2 H, br s, NH and CH=N), 7.47 (2 H, m, Bn-H), 7.36-7.29 (5 H, m, 3 Bn-H and 2 Ar-H [Ts-AA'BB']), 7.03 (1 H, d, J 8.8 Hz, 7/6-H), 6.94 (1 H, d, J 8.8 Hz, 6/7-H), 6.66 (1 H, s, 3-H), 5.19 (2 H, s, OCH_2Ph), 5.05 (3 H, m, $NCH_2CH=CMe_2$ and $CH_2CH=CMe_2$), 3.88 (3 H, s, OMe), 2.40 (3 H, s, Ar-Me), 1.84 (3 H, s, Me), and 1.69 (3 H, s, Me); m/z (FAB, 3-NBA Matrix) 518 (MH^+, 55%), 426 (100), and 242 (20).



(93)

8-Benzyloxy-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole (93)

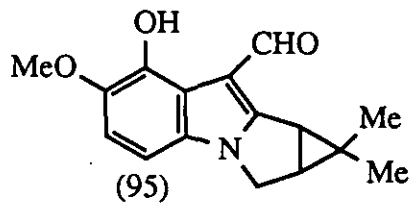
Sodium hydride (80%, 0.032 g, 1.066 mmol) was added to a stirred solution of the tosylhydrazone (92) (0.369 g, 0.714 mmol) in dry THF (11 cm^3). After 20 min, the solution was filtered, and the filtrate evaporated. The residue was dissolved in dry chlorobenzene (105 cm^3), and the solution refluxed for 3.5 h. The solvent was evaporated, and the residue purified by chromatography (gradient elution: 100% light petroleum and going down in 5% intervals to 60% light petroleum : 40% diethyl ether) to give the title compound (93) (0.143 g, 60%) as a brown oil, (Found: M^+, 333.1729. $C_{22}H_{23}NO_2$ requires M, 333.1729); $\nu_{max.}$ ($CHCl_3$) 1570, 1490, 1452, 1432, 1262, and 1232 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 7.56-7.53 (2 H, m, Bn-H), 7.38-7.26 (3 H, m, Bn-H), 6.85-6.78 (2 H, 2d, J 8.6 Hz, 5-H and 6-H), 6.21 (1 H, s, 9-H), 5.22 (2 H, s, OCH_2Ph), 4.13 (1 H, dd, J 6.1, 10.8 Hz, 3-CHH), 3.90 (3 H, s, OMe), 3.87 (1 H, m, 3-CHH), 2.28 (1 H, dd, J 0.5, 6.8 Hz, 1-H), 2.09 (1 H, t, J 7.0 Hz, 2-H), 1.19 (3 H, s, Me), and 0.67 (3 H, s, Me); m/z 334 (MH^+, 8%), 333 (M^+, 31), 242 (100), 200 (13), 91 (17).



(94)

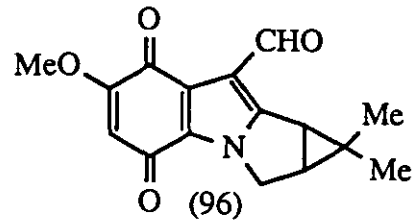
8-Benzyloxy-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde. (94) *N*-Methylformanilide (0.5 cm^3), and phosphorus oxychloride (0.38 cm^3), were stirred under a calcium oxide tube for 10 min. The resulting precipitate was cooled to 0°C. An amount of this yellow precipitate (0.150 g, *N*-methylformanilide 0.070 g, 0.515 mmol, and phosphorus oxychloride 0.080 g, 0.0515 mmol) was added to 8-benzyloxy-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole (93) (0.143 g, 0.429 mmol) in 1,2-dichloroethane (2 cm^3). The mixture was refluxed for 1.25 h. Sodium acetate (1 M, 9 cm^3) was added, and the mixture extracted with ethyl acetate (3 x 50 cm^3). The combined extracts were

washed with water (2 x 50 cm^3), brine (50 cm^3), dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography (eluting with diethyl ether) gave the title compound (94) (0.118 g, 76%) as a yellow oil, (Found: M^+, 361.1678. $C_{23}H_{23}NO_3$ requires M, 361.1678); $\nu_{max.}$ ($CHCl_3$) 1666, 1596, 1526, 1492, 1452, and 696 cm^{-1}, δ_H (250 MHz; $CDCl_3$+TMS) 10.28 (1 H, s, CHO), 7.49-7.15 (5 H, m, Bn-H), 6.87 (2 H, 2d, J 8.6 Hz, 5-H and 6-H), 5.18 (2 H, s, OCH_2Ph), 4.13 (1 H, dd, J 6.4, 11.8 Hz, 3-CHH), 3.91 (3 H, s, OMe), 3.86 (1 H, m, 3-CHH), 2.86 (1 H, d, J 6.3 Hz, 1-H), 2.20 (1 H, t, J 6.3 Hz, 2-H), 1.26 (3 H, s, Me), and 0.68 (3 H, s, Me); m/z 362 (MH^+, 12%), 361 (M^+, 28), 270 (100), 228 (32), and 91 (45).



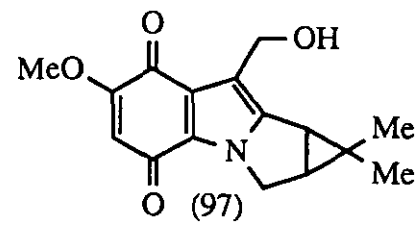
(95)

8-Hydroxy-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde. (95) To a solution of 8-benzyloxy-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde (94) (0.300 g, 0.831 mmol) in ethyl acetate (160 cm^3) was added 10% palladium on carbon catalyst (0.065 g), dilute sulphuric acid (10 drops), and the mixture was stirred under an atmosphere of hydrogen. After 2.5 h the suspension was filtered and washed with dichloromethane (~150 cm^3). The organic layer was extracted with water (3 x 50 cm^3), brine (50 cm^3), dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography (gradient elution:-started with 50% light petroleum : 50% diethyl ether - 100% diethyl ether, in 10% intervals) gave the title compound (95) (0.123 g, 55%) as a colourless solid, m.p. 119-121°C, (Found: M^+, 271.1208. $C_{16}H_{17}NO_3$ requires M, 271.1208); $\nu_{max.}$ ($CHCl_3$) 1606 (br), 1298, and 1252 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.90 (1 H, s, CHO), 9.54 (1 H, s, OH), 6.89 (1 H, d, J 8.5 Hz, 6/5-H), 6.55 (1 H, d, J 8.5 Hz, 5/6-H), 4.18 (1 H, dd, J 6.35, 12.3 Hz, 3-CHH), 3.91 (3 H, s, OMe), 3.86 (1 H, m, 3-CHH), 2.60 (1 H, dd, J 1.4, 6.6 Hz, 1-H), 2.34 (1 H, t, J 6.5 Hz, 2-H), 1.29 (3 H, s, Me), and 0.77 (3 H, s, Me); m/z 273 (MH_2^+, 17%), 272 (MH^+, 57), 271 (M^+, 100).



(96)

9-Formyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione. (96) Potassium nitrosodisulphonate (0.268 g, 1.000 mmol) in water (13 cm^3) was added to a stirred solution of 8-hydroxy-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde (95) (0.123 g, 0.454 mmol) in acetone (44 cm^3). The mixture was buffered with sodium dihydrogen phosphate solution (0.167 M, 13 cm^3). After stirred at room temperature for 12 h, the mixture was concentrated in vacuo, filtered, and the residue recrystallised from dichloromethane-light petroleum to give the title compound (96) (0.111 g, 86%), as orange crystals, m.p. 246-248°C, (Found: M^+, 285.0999. $C_{16}H_{15}NO_4$ requires M, 285.1001); $\nu_{max.}$ ($CHCl_3$) 1678, 1640, 1596, 1506, 1238, and 1136 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.37 (1 H, s, CHO), 5.69 (1 H, s, 6-H), 4.32 (1 H, dd, J 6.4, 14.1 Hz, 3-CHH), 4.18 (1 H, d, J 14.2 Hz, 3-CHH), 3.85 (3 H, s, OMe), 2.71 (1 H, dd, J 1.5, 6.5 Hz, 1-H), 2.22 (1 H, m, 2-H), 1.26 (3 H, s, Me), and 0.72 (3 H, s, Me); m/z 286 (MH^+, 20%), 285 (M^+, 100), and 270 (69).

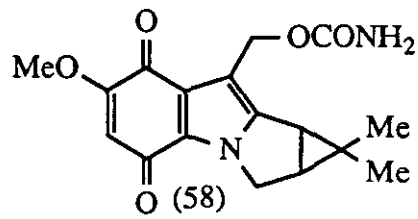


(97)

9-Hydroxymethyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (97)

Sodium borohydride (0.071 g, 1.887 mmol) was added to a stirred solution of 9-formyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (96) (0.078 g, 0.274 mmol), in methanol (53 cm^3), and the solution rigorously degassed with nitrogen. After stirring for 4 h at room temperature under nitrogen, air was blown rapidly through the solution for 5 min. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 100 cm^3). The organic extracts were washed with water (3 x 100 cm^3), brine (100 cm^3), dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography (gradient elution:-

started with 50% ethyl acetate : 50% light petroleum - 100% ethyl acetate) gave the title compound (97) (0.054 g, 69%), as a red solid, m.p. 162-164°C, (Found: M^+, 287.1158. $C_{16}H_{17}NO_4$ requires M, 287.1158); $\nu_{max.}$ ($CHCl_3$) 3468, 1658, 1636, and 1594 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.61 (1 H, s, 6-H), 4.64 (2 H, m, 10-CH_2), 4.28 (1 H, dd, J 6.3, 14.0 Hz, 3-CHH), 4.08 (1 H, d, J 14.0 Hz, 3-CHH), 3.82 (3 H, s, OMe), 2.20 (1 H, d, J 6.9 Hz, 1-H), 2.08 (1 H, t, J 6.5 Hz, 2-H), 1.19 (3 H, s, Me), and 0.72 (3 H, s, Me); m/z 289 (MH_2^+, 8%), 288 (MH^+, 19), and 287 (M^+, 100).



(58)

9-Hydroxymethyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1, 2-a]indole-5,8-dione carbamate (58)

Phenyl chloroformate (0.04 cm^3, 0.28 mmol), was added dropwise to a stirred, ice cold solution of alcohol (97) (0.050 g, 0.174 mmol) in distilled pyridine (11 cm^3). The mixture was stirred at room temperature for 2 h, then water (5 cm^3) was added. The mixture was extracted with dichloromethane (3 x 50 cm^3). The combined extracts were washed with water (3 x 50 cm^3), brine (100 cm^3), and dried ($MgSO_4$). The solvent was evaporated, and the residue purified by column chromatography (eluting with diethyl ether) to give an orange gummy solid.

A solution of the phenyl carbonate (98) in dry dichloromethane (30 cm^3), was cooled to -78°C. Ammonia gas was bubbled into the solution for 30 min (~100 cm^3), after which the contents were allowed to warm to room temperature, and the solvent was evaporated. Recrystallisation of the residue from dichloromethane-light petroleum gave the title compound (58) (0.040 g, 70%) as an orange crystalline solid, m.p. 204-206°C, (Found: M^+, 330.1216. $C_{17}H_{18}N_2O_5$ requires M, 330.1216); $\lambda_{max.}$ (MeOH) 240 (log ε 4.32), 292 (4.21), 348 (3.57), and 474 nm (3.24); $\nu_{max.}$ ($CHCl_3$) 3540, 3428, 1724, 1670, 1636, 1594, 1496, and 1232 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.59 (1 H, s, 6-H), 5.28 (2 H, m, 10-CH_2), 4.59 (2 H, br s, NH_2), 4.28 (1 H, dd, J 6.3, 14.0 Hz, 3-CHH), 4.08 (1 H, d, J 14.0 Hz, 3-CHH), 3.80 (3 H, s, OMe), 2.37 (1 H, d, J 6.9 Hz, 1-H), 2.09 (1 H, t, J 6.0 Hz, 2-H), 1.19 (3 H, s, Me), and 0.69 (3 H, s, Me); m/z 330 (M^+, 15%), 287 (100), 273 (83), 254 (33), 228 (18), and 51 (14).

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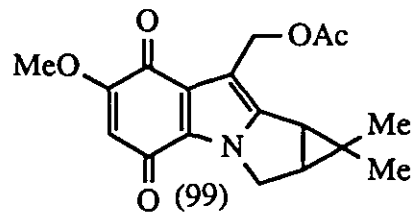
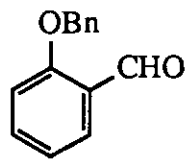


(99)

9-Acetoxymethyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1, 2-a]indole-5,8-dione. (99) To a solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (97) (0.005 g, 0.017 mmol), in distilled pyridine (2 cm^3), was added acetic anhydride (0.5 cm^3). The mixture was stirred at room temperature for 15 h. The mixture was then extracted with dichloromethane (3 x 25 cm^3). The combined extracts were washed with water (3 x 50 cm^3), brine (50 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography (eluting with diethyl ether), to give an orange solid. Recrystallisation of this solid from dichloromethane-light petroleum gave the title compound (99) (0.004 g, 66%), as an orange solid, m.p. 167-169°C, (Found: M^+, 329.1245. $C_{18}H_{19}NO_5$ requires 329.1263); $\nu_{max.}$ ($CHCl_3$) 3008, 1730, 1670, 1636, 1594, and 1228 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.60 (1 H, s, 6-H), 5.26 (2 H, m, 10-CH_2), 4.29 (1 H, dd, J 6.3, 14.0 Hz, 3-CHH); 4.08 (1 H, d, J 14.0 Hz, 3-CHH), 3.80 (3 H, s, OMe), 2.31 (1 H, dd, J 1.2, 7.0 Hz, 1-H), 2.08 (1 H, m, 2-H), 2.06 (3 H, s, CO_2Me), 1.20 (3 H, s, Me), and 0.68 (3 H, s, Me); m/z 329 (M^+, 27%), 287 (100), and 254 (35).

6.4. Experimental For 2.4.

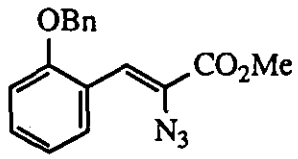


(101)

2-Benzyloxybenzaldehyde (101)

Potassium hydroxide pellets (24.0 g, 428 mmol) were added to a stirred solution of salicylaldehyde (100) (42.0 cm^3, 394 mmol) in absolute ethanol (360 cm^3), followed by benzyl chloride (49.3 cm^3, 428 mmol). The stirred mixture was refluxed for 12 h, then water (300 cm^3)was added, and the mixture extracted with diethyl ether (3 x 350 cm^3). The ethereal extracts were washed with water (2 x 200 cm^3), potassium hydroxide

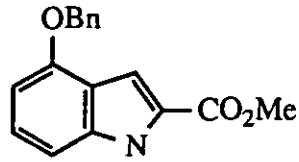
solution (2 M, 5 x 200 cm^3), again with water (2 x 200 cm^3), then brine (200 cm^3). The organic layer was dried ($MgSO_4$), then condensed in vacuo, to give the title compound (101) (53.7 g, 64%), as a colourless solid on trituration with light petroleum, m.p. 37-38 °C (lit.[102] 38°C), (Found: C, 79.1; H, 5.6. $C_{14}H_{12}O_2$ requires C, 79.2; H, 5.7%); $\nu_{max.}$ (Nujol) 1686, 1599, 1484, 1456, 1304, 1287, 1240, and 1162 cm^{-1}; δ_H (270 MHz; $CDCl_3$) 10.57 (1 H, s, CHO), 7.86 (1 H, dd, J 1.9, 7.9 Hz, 6-H), 7.54 (1 H, td, J 1.8, 7.8 Hz, 4-H), 7.47-7.35 (5 H, m, Bn-H), 7.07-7.02 (2 H, m, 3-H and 5-H), and 5.20 (2 H, s, OCH_2Ph); m/z 213 (MH^+, 10%), 212 (M^+, 65), 183 (36), 121 (166), and 91 (100).



(102)

Methyl 2-Azido-3-(2-benzyloxyphenyl)propenoate (102)

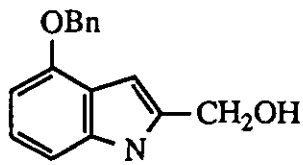
Sodium metal (6.20 g, 269.57 mmol) was added to dry methanol (160 cm^3). The solution was cooled to -15°C, and a solution of methyl azidoacetate (31.00 g, 269.57 mmol) and 2-benzyloxybenzaldehyde (101) (14.36 g, 67.74 mmol) in dry methanol (12 cm^3) was added dropwise by syringe. The mixture was stirred at -10°C for 3 h then at 4°C for 12 h. Water (10 cm^3) was cautiously added to the mixture, which was then extracted with ethyl acetate (2 x 150 cm^3). The combined extracts were washed with water (200 cm^3), brine (150 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a pale yellow residue. The residue was triturated with a small quantity of diethyl ether and the resulting precipitate was filtered off. The remaining oily residue was purified by column chromatography to give the title compound (102) (17.06 g, 82%), as pale luminescent yellow crystals, m.p. 76-78°C, (Found: M^+, 309.1113. $C_{17}H_{15}N_3O_3$ requires M, 309.1114); $\nu_{max.}$ (Nujol) 2121, 1721, 1597, 1451, 1376, 1253, 1113, and 1002 cm^{-1}; δ_H (270 MHz; $CDCl_3$) 8.21 (1 H, dd, J 1.7, 7.8 Hz, 6-H), 7.50 (1 H, s, CH=), 7.45-7.27 (6 H, m, 5 Bn-H and 5/4-H), 7.00 (1 H, t, J 7.0 Hz, 4/5-H), 6.93 (1 H, dd, J 1.0, 8.3 Hz, 3-H), 5.15 (2 H, s, OCH_2Ph), and 3.90 (3 H, s, CO_2Me); m/z 309 (M^+, 11%), 281 (59), 222 (51), 190 (77), 146 (94), and 91 (100).



(103)

Methyl 4-Benzyloxyindole-2-carboxylate (103)

A solution of methyl 2-azido-3-(2-benzyloxyphenyl)propenoate (102) (3.368 g, 10.90 mmol) in dry xylene (80 cm^3) was introduced, dropwise, by means of a pressure equalising dropping funnel, to refluxing dry xylene (320 cm^3). After the addition was complete (ca. 20 min), the solution was refluxed for a further 45 min. After this time, the solvent was evaporated to give a yellow solid residue. The residue was triturated with a small quantity of diethyl ether and the resulting precipitate was filtered off. The remaining oily residue was purified by column chromatography to give the title compound (103) (2.447 g, 80%) as a colourless solid, m.p. 126-128°C, (Found: C, 72.3; H, 5.4; N, 5.0. $C_{17}H_{15}NO_3$ requires C, 72.6; H, 5.4; N, 5.0%); $\nu_{max.}$ (Nujol) 3337, 1696 1582, 1520, 1359, 1243, and 1205 cm^{-1}; δ_H (270 MHz; $CDCl_3$) 8.88 (1 H, s, NH), 7.55-7.31 (6 H, m, 5 Bn-H and 3-H), 7.23 (1 H, t, J 8.0 Hz, 6-H), 7.03 (1 H, d, J 8.3 Hz, 5/7-H), 6.58 (1 H, d, J 7.8 Hz, 7/5-H), 5.22 (2 H, s, OCH_2Ph), and 3.93 (3 H, s, CO_2Me); m/z 282 (MH^+, 17%), 281 (M^+, 90), 190 (35), and 91 (100).

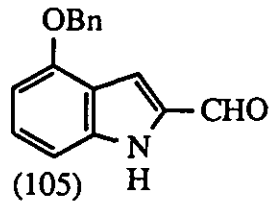


(104)

4-Benzyloxyindole-2-methanol (104)

A solution of methyl 4-benzyloxyindole-2-carboxylate (103) (2.437 g, 8.673 mmol) in dry THF (90 cm^3) was added to a stirred suspension of lithium aluminium hydride (0.646 g, 17.000 mmol) in dry THF (35 cm^3) such that the mixture achieved gentle reflux. After 30 min, water (0.66 cm^3), 15% sodium hydroxide (0.66 cm^3), and again water (2.01 cm^3), were added to the mixture and the resultant precipitate removed by filtration (through a bed of Celite). The filtrate was dried ($MgSO_4$), then condensed in vacuo to give the title compound (104) (1.927 g, 88%) as a colourless solid, m.p. 106-108°C, (Found: C, 76.1; H; 6.0; N, 5.5. $C_{16}H_{15}NO_2$ requires C, 75.9; H, 6.0; N, 5.5%); $\nu_{max.}$ (Nujol) 3465, 3278, 1592, 1511, 1352, 1247, 1022, and 769 cm^{-1}; δ_H (270 MHz; $CDCl_3$) 8.33 (1 H, s, NH), 7.51-7.49 (2 H, m, Bn-H), 7.43-7.30 (3 H, m,

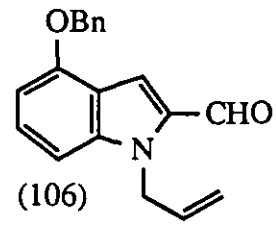
Bn-H), 7.09 (1 H, t, J 7.9 Hz, 6-H), 7.00 (1 H, d, J 8.3 Hz, 5/7-H), 6.59 (2 H, m, 3-H and 7/5-H), 5.22 (1 H, s, OCH_2Ph), 4.81 (2 H, s, CH_2OH), and 1.75 (1 H, s, OH); m/z 254 (MH^+, 11%), 253 (M^+, 55), 162 (100), and 91 (87).



(105)

4-Benzyloxyindole-2-carboxaldehyde (105)

Manganese dioxide (5.810 g, 66.782 mmol) was added, in portions to a stirred solution of 4-benzyloxyindole-2-methanol (104) (1.701 g, 6.723 mmol) in dry dichloromethane (218 cm^3). The suspension was then refluxed for 15 h. The mixture was filtered through a bed of Celite, and the residue washed with hot dichloromethane (500 cm^3). The filtrate and washings were evaporated to give a solid residue which was purified by column chromatography (eluting with dichloromethane) to give the title compound (105) (1.448 g, 86%) as a pale yellow solid, m.p. 164-166°C, (Found: C, 76.4; H, 5.1; N, 5.6. $C_{16}H_{13}NO_2$ requires C, 76.5; H, 5.2; N, 5.6%); $\nu_{max.}$ (Nujol) 3188, 1690, 1668, 1622, 1518, 1250, 1142, and 1082 cm^{-1}; δ_H (270 MHz; $CDCl_3$) 9.79 (1 H, s, CHO), 9.02 (1 H, s, NH), 7.55-7.49 (2 H, m, Bn-H), 7.48 -7.25 (5 H, m, 3 Bn-H, 3-H and 6-H), 7.03 (1 H, d, J 8.3 Hz, 5/7-H), 6.59 (1 H, d, J 7.8 Hz, 7/5-H), and 5.30 (1 H, s, OCH_2Ph); m/z 253 (MH_2^+, 9%), 252 (MH^+, 13), 251 (M^+, 67), and 91 (100).

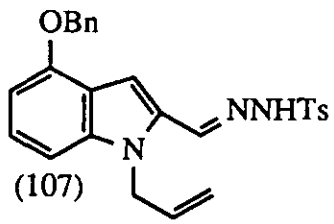


(106)

1-Allyl-4-benzyloxyindole-2-carboxaldehyde (106)

To a flask charged with sodium hydride (80%; 0.211 g, 7.033 mmol) was added dry light petroleum (7 cm^3). The mixture was stirred for 10 min, the petroleum removed by syringe, and the flask contents dried under vacuum. 4-Benzyloxyindole-2-carboxaldehyde (105) (1.422 g, 5.665 mmol) in DMF (112 cm^3) was added dropwise, and the mixture was stirred at room temperature for 45 min. Allyl bromide (0.61 cm^3, 7.058 mmol) was added, and the mixture was stirred at room temperature. After 1.5 h, water (75 cm^3) was cautiously added, and the mixture was extracted with ethyl acetate (3

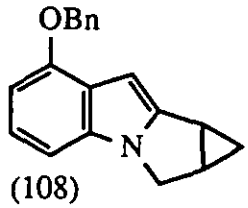
x 150 cm^3). The combined extracts were washed with water (8 x 150 cm^3), brine (150 cm^3), dried ($MgSO_4$), and evaporated to give the title compound (106) (1.628 g, 99%) as a brown oil, (Found: C, 78.2; H, 6.3; N, 4.5. $C_{19}H_{17}NO_2$ requires C, 78.3; H, 5.9; N, 4.8%); $\nu_{max.}$ (Film) 1664, 1612, 1572, 1496, 1454, 1370, 1262, and 1130 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 9.81 (1 H, s, CHO), 7.52-7.28 (7 H, m, 5 Bn-H, 3-H and 6-H), 6.97 (1 H, d, J 8.4 Hz, 5/7-H), 6.59 (1 H, d, J 7.8 Hz, 7/5-H), 5.98 (1 H, m, $CH_2CH=CH_2$), 5.23 (2 H, s, OCH_2Ph), 5.20 (2 H, m, $CH_2CH=CH_2$), 5.11 (1 H, dd, J 1.2, 10.3 Hz, $CH_2CH=CHH$, cis), and 4.90 (1 H, dd, J 1.2, 17.1 Hz, $CH_2CH=CHH$, trans); m/z 292 (MH^+, 6%), 291 (M^+, 27), 200 (16), 91 (100), and 41 (23).



(107)

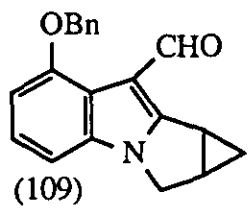
1-Allyl-4-benzyloxyindole-2-carboxaldehyde tosylhydrazone (107)

4-Toluenesulphonyl hydrazide (2.070 g, 11.115 mmol) in dry methanol (33 cm^3) was added to a stirred solution of 1-allyl-4-benzyloxyindole-2-carboxaldehyde (106) (2.622 g, 9.010 mmol) in dry methanol (25 cm^3). After stirring at 40°C for 1 h, the solvent was removed in vacuo, and the residue was triturated with methanol to give the title compound (107) (3.581 g, 87%) as a colourless solid, m.p. 138-139°C, (Found: MH^+, 460.1695. $C_{26}H_{26}N_3O_3S$ requires MH, 460.1695); $\nu_{max.}$ ($CHCl_3$) 3280, 1604, 1576, 1492, 1452, 1360, and 1164 cm^{-1}; δ_H (360 MHz; $CDCl_3$+TMS) 7.83 (2 H, d, J 8.2 Hz, Ar-H [Ts-AA'BB']), 7.76 (1 H, s, CH=N), 7.69 (1 H, br s, NH), 7.48-7.26 (7 H, m, 5 Bn-H and 2 Ar-H [Ts-AA'BB']), 7.17 (1 H, t, J 8.1 Hz, 6-H), 6.90 (1 H, d, J 8.4 Hz, 5/7-H), 6.87 (1 H, s, 3-H), 6.55 (1 H, d, J 7.8 Hz, 7/5-H), 5.85 (1 H, m, $CH=CH_2$), 5.19 (2 H, s, OCH_2Ph), 5.08 (2 H, m, $CH_2CH=CH_2$), 5.03 (1 H, dd, J 1.4, 10.3 Hz, $CH=CHH$, cis), 4.84 (1 H,dd, J 1.4, 17.1 Hz, $CH_2CH=CHH$, trans), and 2.40 (3 H, s, Ar-Me); m/z (FAB, 3-NBA Matrix) 460 (MH^+, 100%), 368 (50), and 91 (87).



(108)

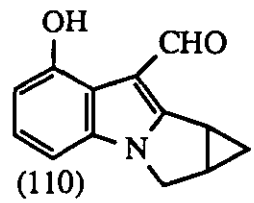
8-Benzyloxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole (108)
Sodium hydride (80%, 0.120 g, 4.000 mmol) was added to a stirred solution of the tosylhydrazone (107) (1.172 g, 2.553 mmol) in dry THF (41 cm^3). After 30 min, the solution was filtered, and the filtrate evaporated. The residue was dissolved in dry chlorobenzene (410 cm^3), and the solution refluxed for 3 h. The solvent was evaporated, and the residue purified by column chromatography (eluting with 50% diethyl ether : 50% light petroleum) to give the title compound (108) (0.677 g, 96%) as a colourless solid, m.p. 117-119°C, (Found: M^+, 275.1310. $C_{19}H_{17}NO$ requires M, 275.1310); $\nu_{max.}$ (Nujol) 1496, 1356, 1262, 1242, and 760 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 7.52-7.48 (2 H, m, Bn-H), 7.42-7.26 (3 H, m, Bn-H), 7.00 (1 H, t, J 8.0 Hz, 6-H), 6.78 (1 H, d, J 8.0 Hz, 7/5-H), 6.54 (1 H, d, J 7.7 Hz, 5/7-H), 6.34 (1 H, s, 9-H), 5.21 (2 H, s, OCH_2Ph), 4.14 (1 H, dd, J 5.3, 10.4 Hz, 3-CHH), 4.04 (1H, d, J 10.4 Hz, 3-CHH), 2.44 (1 H, m, 1-H), 2.35 (1H, m, 2-H), 1.27 (1 H, m, 1a-CHH), and 0.63 (1 H, m, 1a-CHH); m/z 276 (MH^+, 6%), 275 (M^+, 27), 184 (100), 156 (16), and 91 (13).



(109)

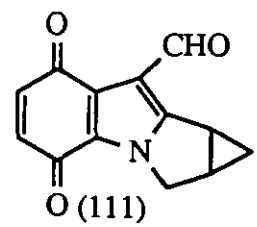
8-Benzyloxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde. (109) *N*-Methylformanilide (0.216 g, 1.598 mmol) and phosphorus oxychloride (0.245 g, 1.598 mmol) were stirred under a calcium chloride drying tube for 10 min. The resulting precipitate was cooled to 0°C, and 1,2-dichloroethane (6 cm^3) was added. 8-Benzyloxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole (108) (0.330 g, 1.200 mmol) was added, and the mixture was refluxed for 1 h. Sodium acetate (1 M, 10 cm^3) was added, and the mixture was extracted with ethyl acetate (3 x 75 cm^3). The combined extracts were washed with water (3 x 100 cm^3), brine (100 cm^3), dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography gave the title compound (109) (0.322 g, 89%) as a colourless solid, m.p. 71-73°C, (Found: C, 79.0;

H, 5.7; N, 4.6. $C_{20}H_{17}NO_2$ requires C, 79.2; H, 5.65; N, 4.6%); $\nu_{max.}$ (film) 2920, 1645, 1535, 1495, 1355, 1255, and 1140 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.43 (1 H, s, CHO), 7.49-7.33 (5 H, m, Bn-H), 7.11 (1 H, t, J 8.0 Hz, 6-H), 6.83 (1 H, d, J 8.0 Hz, 7/5-H), 6.76 (1 H, d, J 8.0 Hz, 5/7-H), 5.24 (2 H, s, OCH_2Ph), 4.17 (2 H, m, 3-CH_2), 3.04 (1 H, m, 1-H), 2.49 (1 H, m, 2-H), 1.50 (1 H, m, 1a-CHH), and 0.71 (1 H, m, 1a-CHH); m/z 304 (MH^+, 6%), 303 (M^+, 28), 212 (100), and 91 (36).



(110)

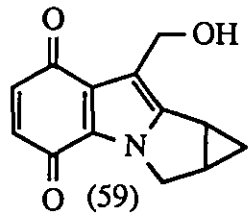
8-Hydroxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde. (110) To a solution of 8-benzyloxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-carboxaldehyde (109) (0.300 g, 0.990 mmol) in ethyl acetate (180 cm^3) was added 10% palladium on carbon catalyst (0.067 g), and dilute sulphuric acid (10 drops), and the mixture stirred under an atmosphere of hydrogen. After 4 h the suspension was filtered and washed with dichloromethane (~150 cm^3). The organic layer was extracted with water (3 x 100 cm^3), brine (100 cm^3), dried ($MgSO_4$), and evaporated. Recrystallisation of the residue from dichloromethane-light petroleum gave the title compound (110) (0.143 g, 68%) as a beige solid, m.p. 119-120°C, (Found: M^+, 213.0790. $C_{13}H_{11}NO_2$ requires M, 213.0790); $\nu_{max.}$ (Nujol) 1616 (br), 1546, 1444, 1300, and 1252 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.67 (1 H, s, CHO), 9.65 (1 H, s, OH), 7.13 (1 H, t, J 8.0 Hz, 6-H), 6.70 (1 H, dd, J 0.7, 8.0 Hz, 7/5-H), 6.61 (1 H, dd, J 0.7, 8.0 Hz, 7/5-H), 4.16 (2 H, m, 3-CH_2), 2.77 (1 H, m, 1-H), 2.59 (1 H, m, 2-H), 1.52 (1 H, m, 1a-CHH), and 0.81 (1 H, m, 1a-CHH); m/z 214 (MH^+, 15%), 213 (M^+, 95), 212 (100), and 51 (35).



(111)

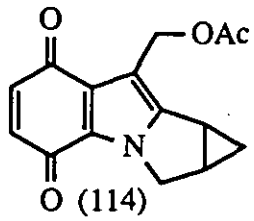
9-Formyl-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (111) Potassium nitrosodisulphonate (0.709 g, 2.642 mmol) in water (36 cm^3) was added to a stirred solution of 8-hydroxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-9-

carboxadehyde (110) (0.262 g, 1.230 mmol) in acetone (120 cm^3). The mixture was buffered with sodium dihydrogen phosphate solution (0.167 M, 36 cm^3). After stirring at room temperature for 12 h, the mixture was concentrated in vacuo, and then filtered. The residue was purified by column chromatography (gradient elution:- 50% diethyl ether : 50% light petroleum - 100% diethyl ether) to give an orange solid. Recrystallisation of the residue from dichloromethane-light petroleum gave the title compound (111) (0.179 g, 64%) as orange crystals, m.p. 162-164°C, (Found: MH^+, 228.0661. $C_{13}H_{10}NO_3$ requires MH, 228.0661); $\nu_{max.}$ (Nujol) 1668, 1646, 1584 1502, 1242, and 1138 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.37 (1 H, s, CHO), 6.60 (2 H, 2d, J 10.5 Hz, 7-H and 6-H), 4.34 (2 H, m, 3-CH_2), 2.88 (1 H, m, 1-H), 2.50 (1 H, m, 2-H), 1.49 (1 H, m, 1a-CHH), and 0.65 (1 H, m, 1a-CHH); m/z 228 (MH^+, 17%), 227 (M^+, 100), 198 (32), and 170 (18).



(59)

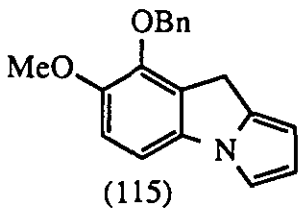
9-Hydroxymethyl-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (59) Sodium borohydride (0.046 g, 1.216 mmol), was added to a stirred solution of 9-formyl-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (111) (0.040 g, 0.176 mmol) in dry methanol (35 cm^3) and the solution was rigorously degassed with nitrogen . After stirring for ***25 min*** (this is very important, longer reactions times lead to decomposition), air was blown through the solution rapidly for 5 min. This mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 75 cm^3). The combined extracts were washed with water (2 x 75 cm^3), brine (75 cm^3), and dried ($MgSO_4$). The solvent was evaporated to give an orange solid which was purified by column chromatography (gradient eluting:-50% diethyl ether : 50% light petroleum - 100% diethyl ether), to give the title compound (59) (0.036 g, 89%), as an orange/red solid, m.p. 106-108°C, (Found: MH^+, 230.0817. $C_{13}H_{12}NO_3$ requires MH, 230.0817); $\nu_{max.}$ ($CHCl_3$) 1634, 1582, 1564, 714 cm^{-1}; δ_H (250 MHz, $CDCl_3$+TMS) 6.53 (2 H, 2d, J 10.5 Hz, 6-H and 7-H), 4.69 (2 H, br s, 10-CH_2); 4.27 (2 H, m, 3-CH_2); 4.05 (1 H, br s, OH), 2.39 (2 H, m, 1-H and 2-H), 1.32 (1 H, m, 1a-CHH), and 0.60 (1 H, m, 1a-CHH); m/z 229 (M^+, 20%), 228 (77), 212 (18), 199 (21), 91 (27), 68 (33), 55 (43), and 39 (100).



(114)

9-Acetoxymethyl-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione.
(114) To a solution of 9-hydroxymethyl-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (59) (0.003 g, 0.013 mmol) in distilled pyridine (1 cm^3), was added acetic anhydride (0.25 cm^3), which was then stirred at room temperature under nitrogen for 15 h. After this time, water (2 cm^3) was added. The mixture was extracted with dichloromethane (3 x 25 cm^3), and the combined extracted were washed with water (3 x 20 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography (eluting with diethyl ether), to give the title compound (114) as an orange solid (0.002 g, 56%), m.p. 137-139°C, (Found: M^+, 271.0845. $C_{15}H_{13}NO_4$ requires M, 271.0845); $\nu_{max.}$ ($CHCl_3$) 3008, 1732, 1648, 1494, 1472, and 1246 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.49 (2 H, m, 6-H and 7-H), 5.29 (2 H, m, 10-CH_2), 4.31 (2 H, m, 3-CH_2), 2.52 (1 H, m, 1-H), 2.38 (1 H, m, 2-H), 2.09 (3 H, s, Me), 1.35 (1 H, m, 1a-CHH), and 0.58 (1 H, m, 1a-CHH); m/z 271 (M^+, 11%), 228 (100), 212 (37), and 43 (25).

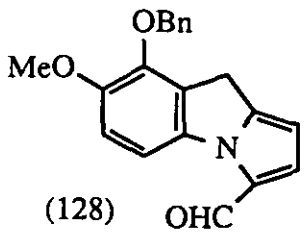
6.5. Experimental For 2.5.



(115)

8-Benzyloxy-7-methoxy-9H-pyrrolo[1,2-a]indole (115)
To a flask charged with sodium hydride (80%, 9.980 mmol, 0.299 g) was added light petroleum (10 cm^3). The mixture was stirred for 10 min, the light petroleum removed by syringe, and the flask contents dried in vacuo. 4-Benzyloxy-5-methoxyindole-2-carboxaldehyde (68) (2.337 g, 8.317 mmol) in dry THF (47 cm^3) was added dropwise and the mixture stirred at room temperature for 30 min (the mixture turned yellow-green in colour). Vinyltriphenylphosphonium bromide (3.685 g, 9.980 mmol) was added, and

the solution was stirred under reflux for 15 h. The salts were removed by filtration through a bed of Celite. The combined filtrate and washings were condensed in vacuo to give a brown solid residue which was purified by column chromatography (eluting with dichloromethane) to give the title compound (115) (2.261 g, 93%) as a colourless solid, m.p. 87-89°C, (Found: C, 78.2; H, 5.8; N, 4.6. $C_{19}H_{17}NO_2$ requires C, 78.3; H, 5.9; N, 4.8%); $\nu_{max.}$ ($CHCl_3$) 2996, 2936, 1482, 1288, and 1264 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 7.46-7.31 (5 H, m, Bn-H), 7.02 (1 H, dd, J 0.8, 2.7 Hz, 3-H), 6.93 (1 H, d, J 8.4 Hz, 5/6-H), 6.86 (1 H, d, J 8.4 Hz, 5/6-H), 6.35 (1 H, t, J 3.1 Hz, 2-H), 6.05 (1 H, m, 1-H), 5.17 (2 H, s, OCH_2Ph), 3.91 (3 H, s, OMe), and 3.66 (2 H, s, 9-CH_2); δ_C (62.9 MHz; $CDCl_3$+TMS) 149.30 (C, 7/8-C), 145.27 (C, 8/7-C), 137.64 (C), 135.86 (C), 135.51 (C), 128.62 (C, Bn-C), 128.45 (CH, Bn-C), 128.35 (CH, Bn-C), 128.17 (CH, Bn-C), 112.63 (CH), 111.67 (CH), 109.53 (CH), 104.40 (CH), 101.46 (CH), 74.57 (CH_2, OCH_2Ph), 56.59 (CH_3, OMe), and 27.15 (CH_2, 9-C); m/z 292 (MH^+, 9%), 291 (M^+, 41), 200 (36), and 91 (100)

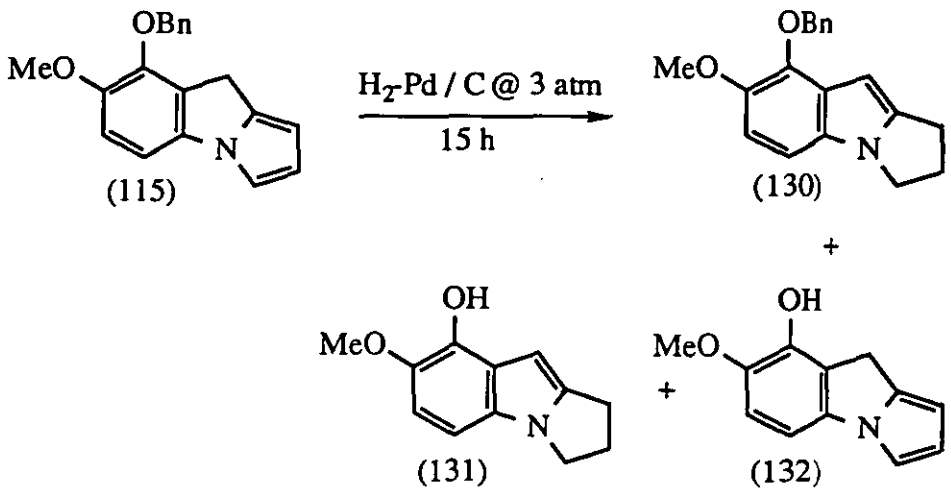


(128)

8-Benzyloxy-7-methoxy-9H-pyrrolo[1,2-a]indole-3-carboxaldehyde (128)

DMF (0.09 cm^3, 1.205 mmol) and phosphorus oxychloride (0.025 cm^3, 0.265 mmol) were stirred in an ice salt bath for 30 min. A solution of 8-benzyloxy-7-methoxy-9H-pyrrolo[1,2-a]indole (115) (0.070 g, 0.241 mmol) in DMF (0.20 cm^3) were added to the DMF / phosphorus oxychloride mixture and the temperature was kept below 0°C. The reaction mixture was then stirred at 35°C for 1 h. After this time, ice water followed by a solution of sodium hydroxide (9.25 M, 1 cm^3) were added and the mixture extracted with dichloromethane (3 x 50 cm^3). The organic layers were washed with water (3 x 50 cm^3), brine (50 cm^3). The organic layers were dried ($MgSO_4$), then condensed in vacuo to give a residue, which was purified by column chromatography (eluting with 50% light petroleum : 50% diethyl ether) to give the title compound (128) (0.029 g, 38%) as a colourless solid, m.p. 132-133°C, (Found: M^+, 319.1219. $C_{20}H_{17}NO_3$ requires M, 319.1208); $\nu_{max.}$ ($CHCl_3$) 2992, 2924, 1658, 1488, 1466, and 1266 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 9.49 (1 H, s, CHO), 8.32 (1 H, d, J 8.7 Hz, 5-H), 7.42-7.31 (5 H, m, Bn-H), 7.09 (1 H, d, J 4.0 Hz, 2-H), 6.93 (1 H, d, J 8.7 Hz, 6-H), 6.20 (1 H, d, J 4.0 Hz, 1-H), 5.14 (2 H, s, OCH_2Ph), 3.93 (3 H, s, OMe), and 3.65 (2 H, s, 9-CH_2); δ_C (100.6 MHz; $CDCl_3$) 177.31 (CH, CHO), 150.06 (C), 145.69 (C), 137.43 (C), 135.33

(C), 128.69 (CH, Bn-C), 128.38 (CH, Bn-C), 128.23 (C),128.15 (CH, Bn-C), 111.58 (CH), 110.96 (CH), 104.50 (CH), 74.53 (CH_2, OCH_2Ph), 56.26 (CH_3, OMe), and 27.89 (CH_2, 9-C); m/z 319 (M^+, 20%), 200 (15), and 91 (100).



7-Methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indol-8-ol (131)

To a solution of 8-benzyloxy-7-methoxy-9H-pyrrolo[1,2-a]indole (115) (0.500 g, 1.718 mmol) in distilled ethyl acetate (150 cm^3) was added 10% palladium on carbon (0.040 g) and the mixture was shaken under an atmosphere of hydrogen (60 psi) for 168 h. (Noting, at least once during the course of the 168 h the catalyst must be filtered off and **new catalyst** must be added to the reaction mixture). After this time the suspension was filtered and the filtrate condensed in vacuo to give a colourless oil. Purification of the residue by column chromatography (gradient elution:-100% light petroleum - 80% light petroleum : 20% diethyl ether) gave the following products: (132) (0.010 g, 3%), (130) (0.030 g, 6%), and (131) (0.349 g, 52%), all as colourless solids.

7-Methoxy-9H-pyrrolo[1,2-a]indol-8-ol (132)

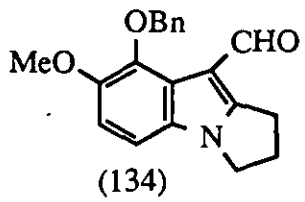
$\nu_{max.}$ ($CHCl_3$) 3532 (sharp), 1486, and 1266 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 7.02 (1 H, m, 3-H), 6.75 (2 H, 2d, J 8.5 Hz, 5-H and 6-H), 6.34 (1 H, t, J 3.0 Hz, 2-H), 6.09 (1 H, m, 1-H), 5.83 (1 H, s, OH), 3.89 (3 H, s, OMe), and 3.80 (2 H, s, 9-CH_2).

8-Benzyloxy-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole (130)

$\nu_{max.}$ ($CHCl_3$) 2992, 1490, 1432, and 1262 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 7.56-7.25 (5 H, m, Bn-H), 6.90 (2 H, 2d, J 8.6 Hz, 5-H and 6-H), 6.20 (1 H, s, 9-H), 5.21 (2 H, s, OCH_2Ph), 4.00 (2 H, t, J 7.0 Hz, 3-CH_2), 3.90 (3 H, s, OMe), 2.98 (2 H, t, J 7.4 Hz, 1-CH_2), and 2.58 (2 H, q, J 7.2 Hz, 2-CH_2); δ_C (62.90 MHz; $CDCl_3$+TMS) 145.08 (C, 7/8-C), 145.02 (C, 8/7-C), 140.80 (C), 138.57 (C), 130.24 (C), 128.38

(Bn), 128.30 (Bn), 128.01 (Bn), 127.93 (Bn), 127.67 (Bn), 110.14 (CH, 5/6-C), 104.68 (CH, 6/5-C), 89.63 (CH, 9-C), 74.91 (CH_2, OCH_2Ph), 58.60 (CH_3, OMe), 43.83 (CH_2, 3-C), 27.88 (CH_2, 1-C), and 24.43 (CH_2, 2-C).
Note: Bn - impossible to pick out in this region whether or not the peaks are due to C or CH.

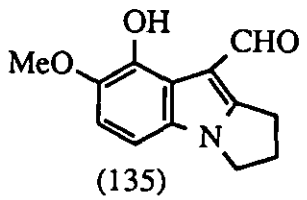
7-Methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indol-8-ol (131)
m.p. 123-124°C, (Found: M^+, 203.0946. $C_{12}H_{13}NO_2$ requires M, 203.0946); $\nu_{max.}$ ($CHCl_3$) 3528 (sharp), 2952, 1494, 1452, and 1254 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.82 (1 H, d, J 8.6 Hz, 5/6-H), 6.70 (1 H, d, J 8.6 Hz, 6/5-H), 6.21 (1 H, s, 9-H), 5.82 (1 H, s, OH), 3.98 (2 H, t, J 7.0 Hz, 3-CH_2), 3.89 (3 H, s, OMe), 2.98 (2 H, t, J 7.4 Hz, 1-CH_2), and 2.59 (2 H, q, J 7.5 Hz, 2-CH_2); δ_C (62.90 MHz; $CDCl_3$+TMS) 144.66 (C, 8-C), 138.68 (C), 138.00 (C), 130.40 (C), 122.38 (C), 107.73 (CH, 5/6-C), 100.56 (CH, 6/5-C), 88.92 (CH, 9-C), 58.43 (CH_3, OMe), 43.72 (CH_2, 3-C), 27.93 (CH_2, 1-C), and 24.38 (CH_2, 2-C); m/z 204 (MH^+, 9%), 203 (M^+, 62), and 188 (100).



(134)

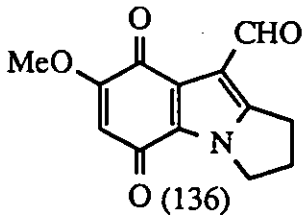
8-Benzyloxy-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-9-carboxaldehyde (134)
N-Methylformanilide (0.5 cm^3) and phosphorus oxychloride (0.38 cm^3) were stirred under a calcium oxide drying tube for 10 min. This precipitate was cooled to 0°C. An amount of the resulting precipitate (0.110 g; *N*-methylformanilide, 0.052 g, 0.381 mmol, and phosphorus oxychloride, 0.058 g, 0.381 mmol) was added to 8-benzyloxy-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole (130) (0.093 g, 0.317 mmol) in distilled 1,2-dichloroethane (2 cm^3). The mixture was refluxed for 1.25 h. Sodium acetate (1 M, 10 cm^3) was added and the mixture was extracted with ethyl acetate (3 x 75 cm^3). The combined organic extracts were washed with water (3 x 75 cm^3), brine (75 cm^3), dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography (eluting with diethyl ether) gave the title compound (134) (0.060 g, 59%), as a colourless solid, m.p. 100-102°C, (Found: M^+, 321.1370. $C_{20}H_{19}NO_3$ requires M, 321.1365); $\nu_{max.}$ ($CHCl_3$) 2988, 1636, 1520, 1490, and 1256 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.32 (1 H, s, CHO), 7.49-7.25 (5 H, m, Bn-H), 6.93 (2 H, 2d, J 8.6 Hz, 5-H and 6-H), 5.19 (2 H, s, OCH_2Ph), 4.03 (2 H, t, J 7.2 Hz, 3-CH_2),

3.96 (3 H, s, OMe), 3.29 (2 H, t, J 7.55 Hz, 1-CH_2), and 2.63 (2 H, q, J 7.4 Hz, 2-CH_2); δ_C (62.90 MHz; $CDCl_3$+TMS) 186.71 (CH, CHO), 153.20 (C), 147.97 (C), 141.43 (C), 137.56 (C), 129.52 (C, Bn-C), 128.43 (CH, Bn-C), 128.03 (CH, Bn-C), 125.77 (C), 110.39 (CH, 5/6-C), 110.00 (C), 105.87 (CH, 6/5-H), 74.82 (CH_2, OCH_2Ph), 57.68 (CH_3, OMe), 44.52 (CH_2, 3-C), and 26.62 (2 x CH_2, 1-C and 2-C); m/z 322 (MH^+, 4%), 321 (M^+, 18), 230 (100), and 91 (20).



(135)

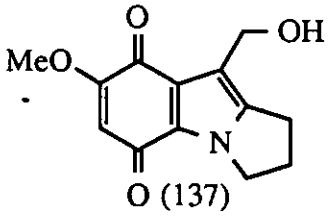
8-Hydroxy-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-9-carboxyaldehyde (135) DMF (0.64 cm^3, 8.250 mmol) and phosphorus oxychloride (0.17 cm^3, 1.815 mmol) were stirred in an ice salt bath for 30 min. 7-Methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indol-8-ol (131) (0.335 g, 1.650 mmol) in DMF (0.19 cm^3, 2.475 mmol) were added to the DMF / phosphorus oxychloride mixture and the temperature was kept below 0°C. The reaction mixture was then stirred at 35°C for 1 h. After this time, ice water followed by a solution of sodium hydroxide (9.25 M, 8 cm^3) were added and the mixture extracted with dichloromethane (3 x 100 cm^3). The organic layers were washed with water (3 x 75 cm^3), brine (100 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a residue, which was purified by column chromatography (eluting with ethyl acetate) to give the title compound (135) (0.277 g, 73%) as a pale yellow solid, m.p. 161-162°C, (Found: M^+, 231.0895. $C_{13}H_{13}NO_3$ requires M, 231.0895); $\nu_{max.}$ ($CHCl_3$) 1606, 1296, and 1252 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.82 (1 H, s, CHO), 9.17 (1 H, s, OH), 6.82 (1 H, d, J 8.6 Hz, 5/6-H), 6.50 (1 H, d, J 8.5 Hz, 6/5-H), 3.95 (2 H, t, J 7.2 Hz, 3-CH_2), 3.89 (3 H, s, OMe), 3.07 (2 H, t, J 7.6 Hz, 1-CH_2), and 2.66 (2 H, q, J 7.4 Hz, 2-CH_2); δ_C (62.90 MHz; $CDCl_3$+TMS) 182.88 (CH, CHO), 158.00 (C, 8/7-C), 142.81 (C, 7/8-C), 140.92 (C), 130.04 (C), 119.58 (C), 111.75 (CH, 5/6-C), 110.39 (C), 100.60 (CH, 6/5-C), 57.58 (CH_3, OMe), 44.94 (CH_2, 3-C), 26.24 (CH_2, 1-C), and 24.40 (CH_2, 2-C); m/z 232 (MH^+, 10%), 231 (M^+, 65), 216 (80), 188 (19), and 86 (100).



(136)

9-Formyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (136)

Potassium nitrosodisulphonate (1.117 g, 4.167 mmol) in water (56 cm^3) was added to a stirred solution of 8-hydroxy-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-9-carboxyaldehyde (135) (0.437 g, 1.892 mmol) in acetone (185 cm^3). The mixture was buffered with sodium dihydrogen phosphate solution (0.167 M, 56 cm^3). After stirring at room temperature for 15 h, the acetone was removed in vacuo. The aqueous layer was then extracted with dichloromethane (3 x 75 cm^3). The organic extracts were washed with water (3 x 50 cm^3), brine (50 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was recrystallised from dichloromethane-light petroleum to give the title compound (136) (0.415 g, 89%) as an orange solid, m.p. 224-225°C (lit.[103] 247-248°C), (Found: M^+, 245.069. $C_{13}H_{11}NO_4$ requires M, 245.069); $\nu_{max.}$ ($CHCl_3$) 1668, 1638, 1596, 1506, and 1124 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 10.36 (1 H, s, CHO), 5.70 (1 H, s, 6-H), 4.29 (2 H, t, J 7.4 Hz, 3-CH_2), 3.86 (3 H, s, OMe), 3.15 (2 H, t, J 7.6 Hz, 1-CH_2), and 2.65 (2 H, q, J 7.5 Hz, 2-CH_2); δ_C (62.90 MHz; $CDCl_3$+TMS) 186.86 (CH, CHO), 178.20 (CO, 8-C), 177.41 (CO, 5-C), 160.65 (C, 7-C), 149.41 (C, 4a-C), 127.06 (C, 9a/8a-C), 125.22 (C, 8a/9a-C), 116.07 (C, 9-C), 105.55 (CH, 6-C), 56.74 (CH_3, OMe), 47.40 (CH_2, 3-C), 26.89 (CH_2, 1-C), and 25.16 (CH_2, 2-C); m/z 246 (MH^+, 15%), and 245 (M^+, 100%).

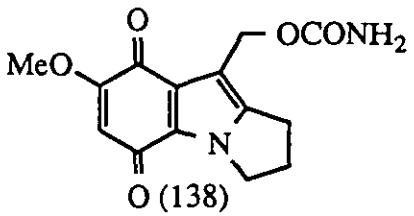


(137)

9-Hydroxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (137)

Sodium borohydride (0.454 g, 12.001 mmol) was added to a stirred solution of 9-formyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (136) (0.421 g, 1.718 mmol) in distilled methanol (443 cm^3) which was rigorously degassed with nitrogen. After stirring for 4 h at room temperature, air was rapidly blown through the solution, which was transferred to a separating funnel. The solution was extracted with

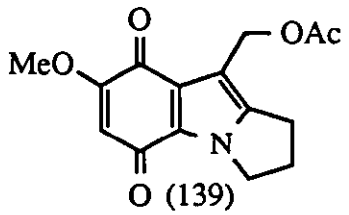
dichloromethane (3 x 300 cm^3). The combined extracts were washed with water (3 x 200 cm^3), brine (200 cm^3), dried ($MgSO_4$). The solvent was then removed in vacuo. Purification of the residue by column chromatography (eluting with ethyl acetate) gave the title compound (137) (0.274 g, 65%) as a red solid, m.p. 154-156°C, (Found: M^+, 247.0839. $C_{13}H_{13}NO_4$ requires M, 247.0844); $\nu_{max.}$ ($CHCl_3$) 3528, 1636, and 1594 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 5.62 (1 H, s, 6-H), 4.59 (2 H, d, J 7.0 Hz, 10-CH_2), 4.21 (2 H, t, J 7.3 Hz, 3-CH_2), 3.91 (1 H, t, J 7.0 Hz, OH), 3.82 (3 H, s, OMe), 2.83 (2 H, t, J 7.4 Hz, 1-CH_2), and 2.56 (2 H, q, J 7.3 Hz, 2-CH_2); δ_C (62.90 MHz; $CDCl_3$+TMS) 179.00 (CO, 8-C), 177.83 (CO, 5-C), 160.54 (C, 7-C), 141.82 (C, 4a-C), 127.03 (C, 9a/8a-C), 125.18 (C, 8a/9a-C), 117.95 (C, 9-C), 105.99 (CH, 6-C), 56.63 (CH_2O/OMe, 10-C/OMe), 56.54 (OMe/OCH_2, MeO/10-C), 46.97 (CH_2, 3-C), 27.40 (CH_2, 1-C), and 22.52 (CH_2, 2-C); m/z 248 (MH^+, 16%), 247 (M^+, 100), 232 (51), and 51 (36).



9-Hydroxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione carbamate. (138) To a stirred cooled solution 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (137) (0.120 g, 0.486 mmol) in anhydrous pyridine (31 cm^3) at 0°C was added phenyl chloroformate (0.09 cm^3, 0.729 mmol) dropwise. The solution was allowed to warm up to room temperature and then stirred for a further 2 h. The solution was extracted with dichloromethane (3 x 75 cm^3). The combined extracts were washed with water (3 x 75 cm^3), brine (100 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography (eluting with ethyl acetate) to give an orange gummy solid.

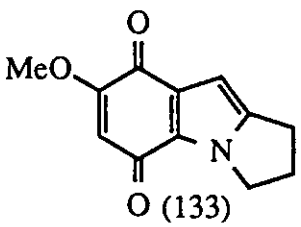
Ammonia gas was bubbled through a solution of the phenyl carbonate in distilled dichloromethane (60 cm^3) at -78 °C and the reaction was monitored by TLC. After approximately 40 min all the starting material had disappeared and the ammonia gas was allowed to evaporate off at room temperature. Then the contents were condensed in vacuo. Recrystallisation of the residue from dichloromethane-light petroleum gave the title compound (138) (0.096 g, 68%) as an orange solid, m.p. 221-223°C, (Found: M^+, 290.0901. $C_{14}H_{14}N_2O_5$ requires M, 290.0903); $\lambda_{max.}$ (MeOH) (qualitative) 228, 289, 348, and 452 nm; $\nu_{max.}$ (Nujol) 3368, 3222, 1773, 1670, 1625, 1585, 1499, 1399, and

1084 cm^{-1}; δ_H (250 MHz; DMSO) 6.51 (2 H, br s, NH_2), 5.75 (1 H, s, 6-H), 5.01 (2 H, s, 10-CH_2), 4.13 (2 H, t, J 7.2 Hz, 3-CH_2), 3.75 (3 H, s, OMe), 2.83 (2 H, t, J 7.4 Hz, 1-CH_2), and 2.52-2.40 (2 H, q [which is covered by DMSO peak], 2-CH_2); m/z 290 (M^+, 17%), 247 (100), and 229 (92).



(139)

9-Acetoxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (139)
Distilled acetic anhydride (0.5 cm^3) was added to a stirred solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (137) (0.015 g, 0.061 mmol) in anhydrous pyridine (3 cm^3). After 2.5 h water (5 cm^3) was added, and extracted with dichloromethane (3 x 50 cm^3). The organic extracts were washed with water (3 x 50 cm^3), brine (75 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave an orange residue, which was recrystallised from dichloromethane-light petroleum to give the title compound (138) (0.014 g, 78%) as an orange solid, m.p. 216-218°C, (Found: M^+, 289.095. $C_{15}H_{15}NO_5$ requires M, 289.095); $\lambda_{max.}$ (MeOH) 227 (log ε 4.57), 286 (4.58), 348 (3.85), and 456 nm (3.52); $\nu_{max.}$ ($CHCl_3$) 3023, 1739, 1673, 1639, 1597, 1501, and 1233 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 5.61 (1 H, s, 6-H), 5.22 (2 H, s, 10-CH_2), 4.23 (2 H, t, J 7.3 Hz, 3-CH_2), 3.80 (3 H, s, OMe), 2.89 (2 H, t, J 7.5 Hz, 1-CH_2), 2.56 (2 H, q, J 7.4 Hz, 2-CH_2), and 2.06 (3 H, s, OCOMe); m/z 289 (M^+, 11%), 247 (100), 230 (46), and 43 (39).

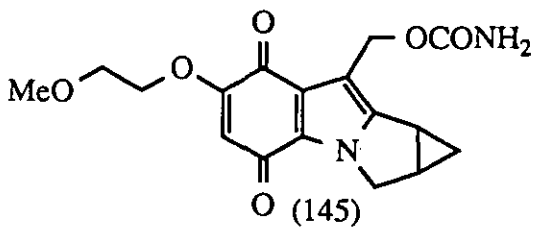


(133)

7-Methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (133)
Potassium nitrosodisulphonate (0.311 g, 1.160 mmol) in water (15 cm^3) was added to a stirred solution of 7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indol-8-ol (131) (0.107 g, 0.527 mmol) in acetone (50 cm^3). The mixture was buffered with sodium dihydrogen phosphate solution (0.167 M, 15 cm^3). After stirring at room temperature for 15 h, the

acetone was removed in vacuo. The aqueous layer was then extracted with dichloromethane (3 x 75 cm^3). The organic extracts were washed with water (3 x 50 cm^3), brine (75 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography (eluting with ethyl acetate) to give an orange solid. The orange residue was recrystallised from dichloromethane-light petroleum to give the title compound (133) (0.065 g, 57%) as an orange solid, m.p. 186.5°C (decomp.), (Found: M^+, 217.0735. $C_{12}H_{11}NO_3$ requires M, 217.0739); $\lambda_{max.}$ (MeOH) 226 (log ε 4.33), 288 (4.24), and 445 nm (3.09); $\nu_{max.}$ ($CHCl_3$) 3024, 1673, 1642, 1596, 1476, 1239, 1154 and 1083 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.29 (1 H, s, 9-H), 5.61 (1 H, s, 6-H), 4.23 (2 H, t, J 7.2 Hz, 3-CH_2), 3.84 (3 H, s, OMe), 2.86 (2 H, t, J 7.4 Hz, 1-CH_2), and 2.58 (2 H, q, J 7.2 Hz, 2-CH_2); δ_C (62.90 MHz; $CDCl_3$+TMS) 178.00 (CO, 8-C), 177.35 (CO, 5-C), 160.67 (C, 7-C), 144.81 (C, 4a-C), 127.84 (C, 9a/8a-C), 126.68 (C, 8a/9a-C), 105.79 (CH, 6-C), 99.98 (CH, 9-C), 56.52 (CH_3, OMe), 46.86 (CH_2, 3-C), 27.61 (CH_2, 1-C), and 23.61 (CH_2, 2-C); m/z 218 (MH^+, 13%), and 217 (M^+, 100).

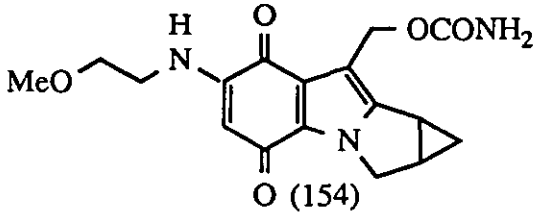
6.6. Experimental For Chapter 3



(145)

9-Hydroxymethyl-7-(2-methoxyethoxy)-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate. (145) A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57) (0.010 g, 0.033 mmol) in dry 2-methoxyethanol (1 cm^3) was stirred under nitrogen for 1 h with 1.6% solution of potassium hydroxide in the alcohol (0.024 g). The mixture was neutralized with excess carbon dioxide while the reaction flask was immersed in a water bath at room temperature. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (2 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography (ethyl acetate) to give an orange solid. Recrystallisation of this orange solid from dichloromethane-light petroleum gave the title compound (145) (0.008 g, 70%) as an

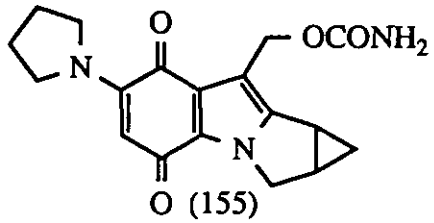
orange solid, m.p. 214-216°C, (Found: M$^+$, 346.1170. $C_{17}H_{18}N_2O_6$ requires M, 346.1165); $\nu_{max.}$ ($CHCl_3$) 3540, 3426, 1770, 1674, 1593, 1555, 1141, 1130, 1107, and 1080 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.60 (1 H, s, 6-H), 5.28 (2 H, m, 10-CH_2), 4.62 (2 H, br s, NH_2), 4.28 (2 H, m, 3-CH_2), 4.03 (2 H, m, 1'-CH_2), 3.80 (2 H, m, 2'-CH_2), 3.45 (3 H, s, OMe), 2.54 (1 H, m, 1-H), 2.36 (1 H, m, 2-H), 1.30 (1 H, 1a-CHH), and 0.59 (1 H, m, 1a-CHH); m/z 346 (M$^+$, 12%), 303 (34), 254 (12), 240 (24), 227 (28), 59 (58), 45 (100), and 44 (99).



(154)

9-Hydroxymethyl-7-(2-methoxyethylamino)-1,2-dihydro-3H-1,2-cyclopropapyrrolo [1,2-a]indole-5,8-dione carbamate (154)

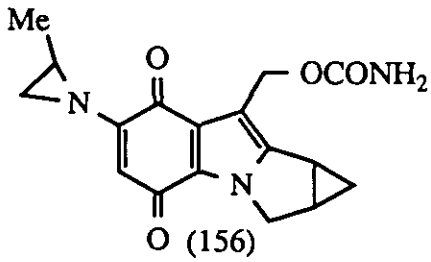
A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57) (0.012 g, 0.040 mmol) in dry DMF (2.5 cm^3) was treated with an excess of 2-methoxyethylamine (0.5 cm^3), and this mixture was stirred at room temperature under nitrogen for 15 h. After this time, water was added (5 cm^3), and extracted with dichloromethane (3 x 25 cm^3). The dichloromethane extracts were washed with water (4 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a purple solid, which was purified by chromatography, (eluting ethyl acetate) to give a purple solid. Recrystallisation of this residue from dichloromethane-light petroleum gave the title compound (154) (0.009 g, 64%), as a purple solid, m.p. 216-218°C, (Found: M$^+$ 345.1318. $C_{17}H_{19}N_3O_5$ requires M, 345.1325); $\lambda_{max.}$ (MeOH) 215 (log ε 4.29), 247 (4.39), 317 (4.14), and 537 nm (3.13); $\nu_{max.}$ ($CHCl_3$) 3544, 3424, 3368, 1722, 1662, 1610, 1588, 1288, and 1262 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.20 (1 H, br s, NH), 5.27 (2 H, m, 10-CH_2), 5.11 (1 H, s, 6-H), 4.61 (2 H, br s, NH_2), 4.27 (2 H, m, 3-CH_2), 3.59 (2 H, t, J 5.2 Hz, 2'-CH_2), 3.38 (3 H, s, OMe), 3.28 (2 H, m, 1'-CH_2NH), 2.48 (1 H, m, 1-H), 2.32 (1 H, m, 2-H) 1.30 (1 H, m, 1a-CHH), and 0.56 (1 H, m, 1a-CHH); m/z 345 (M$^+$, 16%), 239 (100), and 44 (47).



9-Hydroxymethyl-7-(pyrrolidin-1-yl)-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (155)

A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57) (0.010 g, 0.033 mmol) in dry DMF (1.5 cm^3) was treated with distilled pyrrolidine (0.008 cm^3, 0.099 mmol), and this mixture was stirred at room temperature under nitrogen for 15 h. After this time, water was added (5 cm^3), and extracted with dichloromethane (3 x 25 cm^3). The dichloromethane extracts were washed with water (5 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a purple residue, which was recrystallised from dichloromethane-light petroleum to give the title compound (155) (0.008 g, 73%) as a purple solid, m.p. 210°C (decomp.), (Found: M^+, 341.1376. $C_{18}H_{19}N_3O_4$ requires M, 341.1376); $\lambda_{max.}$ (MeOH) (qualitative) 247, 328, and 549 nm; $\nu_{max.}$ ($CHCl_3$) 3612, 3408, 1726, 1686, 1608, 1544, 1498, and 1228 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS at 318 K) 5.31 (2 H, m, 10-CH_2) 5.19 (1 H, s, 6-H), 4.55 (2 H, br s, NH_2), 4.29 (2 H, m, 3-CH_2), 3.64* (4 H, br m, 2 x CH_2N [pyrrolidine ring]), 2.49 (1 H, m, 1-H), 2.30 (1 H, m, 2-H), 1.95 (4 H, m, 2 x CH_2 [pyrrolidine ring]), 1.27 (1 H, m, 1a-C<u>H</u>H), and 0.55 (1 H, m, 1a-CH<u>H</u>); δ_C (100.6 MHz; $CDCl_3$) 180.25 (CO, 8-C), 176.63 (CO, 5-C), 159.47 (C/CO, 7-C/urethane), 156.62 (CO/C, urethane/7-C), 144.49 (C, 4a-C), 128.43 (C, 9a/8a-C), 122.91 (8a/9a-C), 109.75 (9-C), 100.16 (CH, 6-C), 58.36 (CH_2, 10-C), 51.21 (CH_2, 2 x CH_2N [pyrrolidine ring]), 49.73 (CH_2, 3-C), 29.60 (CH_2, 2 x CH_2-pyrrolidine ring), 20.68 (1-C), 16.24 (CH_2, 1a-C), and 14.40 (CH, 2-C); m/z 341 (M^+, 4%), 279 (52), 91 (30), and 44 (100).

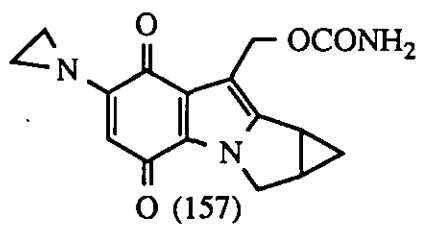
Note: On heating to 318 K, the peak at 3.64 ppm went from a broad peak to a sharper multiplet at this temperature.



(156)

9-Hydroxymethyl-7-(2-methylaziridin-1-yl)-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (156)

A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57) (0.010 g, 0.033 mmol) in dry DMF (1.5 cm^3) was treated with an excess of 2-methylaziridine (0.25 cm^3 initially and 0.25 cm^3 24 h later), and this mixture was stirred at room temperature under nitrogen for 48 h. After this time, water (5 cm^3) was added, and extracted with dichloromethane (3 x 25 cm^3). The dichloromethane extracts were washed with water (5 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a red residue, which was purified by column chromatography (eluting with ethyl acetate) to give a red solid. Recrystallisation of the red residue from dichloromethane-light petroleum gave the title compound (156) (0.0075 g, 68%) as a red solid, m.p. 176-178°C, (Found: M^+, 327.1219. $C_{17}H_{17}N_3O_4$ requires M, 327.1219); $\lambda_{max.}$ (MeOH) 240 (log ε 4.34), 314 (4.17), and 499 nm (3.29); $\nu_{max.}$ ($CHCl_3$) 3544, 3424, 1724, 1662, 1630, 1580, 1496, 1338, and 1256 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.70 (1 H, s, 6-H) 5.29 (2 H, m, 10-CH_2), 4.64 (2 H, br s, NH_2), 4.25 (2 H, m, 3-CH_2), 2.52 (1 H, m, 1-H), 2.27 (2 H, m, 2-H and MeCH-N), 2.08 (2 H, m, CH_2N [aziridine]), 1.42 (3 H, d, J 5.5 Hz, Me), 1.30 (1 H, m, 1a-CHH), and 0.55 (1 H, m, 1a-CHH); m/z 328 (MH^+, 20%), 327 (M^+, 100).

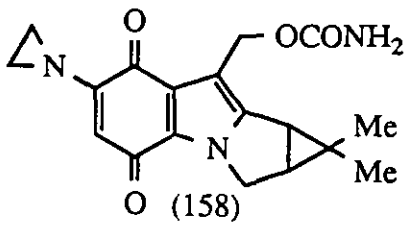


(157)

7-(Aziridin-1-yl)-9-hydroxymethyl-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (157)

A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57) (0.015 g, 0.050 mmol) in dry DMF (2.5 cm^3) was treated with an excess of distilled aziridine (0.5 cm^3), and this mixture was stirred at

room temperature under nitrogen for 15 h. After this time, water (5 cm^3) was added, and extracted with dichloromethane (3 x 25 cm^3). The dichloromethane extracts were washed with water (6 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a red residue, which was recrystallised from dichloromethane-light petroleum to give the title compound (157) (0.013 g, 84%) as a red solid, m.p. 184°C (decomp.), (Found: M^+, 313.1063. $C_{16}H_{15}N_3O_4$ requires M, 313.1063); $\lambda_{max.}$ (MeOH) 239 (log ε 4.45), 308 (4.30), and 490 nm (3.39); $\nu_{max.}$ ($CHCl_3$) 3532, 3424, 1724, 1662, 1632, and 1582 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 5.72 (1 H, s, 6-H) 5.27 (2 H, AB, J 12.5 Hz, 10-CH_2), 4.62 (2 H, br s, NH_2), 4.24 (2 H, m, 3-CH_2), 2.53 (1 H, m, 1-H), 2.32 (1 H, m, 2-H), 2.18 (4 H, s, 2 x CH_2N [aziridine]), 1.29 (1 H, m, 1a-CHH), and 0.54 (1 H, m, 1a-CHH); δ_C (100.6 MHz; $CDCl_3$) 179.08 (CO, 8-C), 177.48 (CO, 5-C), 157.73 (C/CO, 7-C/urethane), 156.77 (CO/C, urethane/7-C), 146.16 (C, 4a-C), 127.40 (C, 9a/8a-C), 126.50 (C, 8a/9a-C), 115.57 (CH, 6-C), 111.20 (C, 9-C), 57.85 (CH_2, 10-C), 49.90 (CH_2, 3-C), 27.51 (CH_2, CH_2N [aziridine]), 20.59 (CH, 1-C), 16.16 (CH_2, 1a-C), and 14.65 (CH, 2-C); m/z 313 (M^+, 16%), 270 (27), 251 (17), and 41 (100).

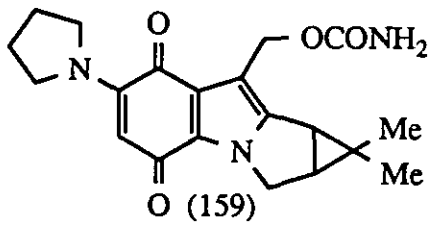


(158)

7-(Aziridin-1-yl)-9-hydroxymethyl-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (158)

A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (58) (0.010 g, 0.030 mmol) in distilled DMF (1.5 cm^3) was treated with distilled aziridine (0.004 g, 0.091 mmol), and the mixture was stirred at room temperature under nitrogen for 48 h. After this time, water (5 cm^3) was added, and extracted with dichloromethane (3 x 25 cm^3). The dichloromethane extracts were washed with water (8 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a red residue, which was recrystallised from dichloromethane-light petroleum to give the title compound (158) (54) (0.0075 g, 75%), as a red solid, m.p. 169-171°C, (Found: M^+, 341.1376. $C_{18}H_{19}N_3O_4$ requires M, 341.1376); $\lambda_{max.}$ (MeOH) (qualitative) 241, 313, 495 nm; $\nu_{max.}$ ($CHCl_3$) 3540, 3430, 1725, 1665, 1630, 1580, 1335, and 1255 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.73 (1 H, s, 6-H), 5.27 (2 H, m, 10-CH_2), 4.69 (2 H, br s,

NH_2), 4.26 (1 H, dd, J 6.3, 14.0 Hz, 3-CHH), 4.06 (1 H, d, J 14.0 Hz, 3-CHH), 2.37 (1 H, dd, J 1.2, 6.9 Hz, 1-H), 2.19 (4 H, s, 2 x CH_2N [aziridine ring]), 2.09 (1 H, t, J 6.5 Hz, 2-H), 1.19 (3 H, s, 1a-CCH_3CH_3), and, 0.68 (3 H, s, 1a-CCH_3CH_3); m/z 341 (M^+, 14%), 298 (22), 265 (14), 91 (7), and 44 (100).

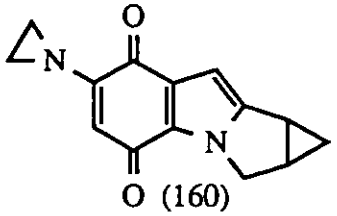


(159)

9-Hydroxymethyl-7-(pyrrolidin-1-yl)-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo [1,2-a]indole-5,8-dione carbamate (159)

A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-1a,1a-dimethyl-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (58) (0.010 g, 0.030 mmol) in distilled DMF (1.5 cm^3) was treated with pyrrolidine (0.008 cm^3, 0.091 mmol), and the mixture was stirred at room temperature under nitrogen for 15 h. After this time, water (5 cm^3) was added, and extracted with dichloromethane (3 x 25 cm^3). The dichloromethane extracts were washed with water (8 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a purple residue, which was purified by column chromatography (gradient elution: 50% ethyl acetate : 50% light petroleum - 100% ethyl acetate) to give a purple residue. Recrystallisation of the purple residue from dichloromethane-light petroleum gave the title compound (159) (0.008 g, 73%), as a purple solid, m.p. 202°C (decomp.), (Found: M^+, 369.1688. $C_{20}H_{23}N_3O_4$ requires M, 369.1688); $\lambda_{max.}$ (MeOH) 254 (log ε 4.40), 330 (4.06), and 549 nm (3.52); $\nu_{max.}$ ($CHCl_3$) 3540, 3424, 1724, 1662, 1602, 1544, 1498, and 1266 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.28 (2 H, m, 10-CH_2), 5.17 (1 H, s, 6-H), 4.59 (2 H, br s, NH_2), 4.29 (1 H, dd, J 6.3, 14.0 Hz, 3-CHH), 4.10 (1 H, d, J 14.0 Hz, 3-CHH), 3.59 (4 H, br s, 2 x CH_2N [pyrrolidine ring])*, 2.33 (1 H, d, J 6.9, 1-H), 2.04 (1 H, t, J 5.9 Hz, 2-H), 1.94 (4 H, m, 2 x CH_2 [pyrrolidine ring]), 1.17 (3 H, s, 1a-CCH_3CH_3), and 0.67 (3 H, s, CCH_3CH_3); m/z 369 (M^+, 9%), 308 (100), and 293 (81).

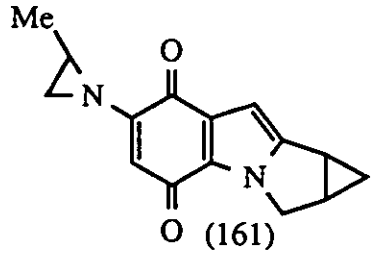
Note: * On heating to 318 K the peak at 3.59 ppm went from a broad peak to a sharper multiplet at this higher temperature.



(160)

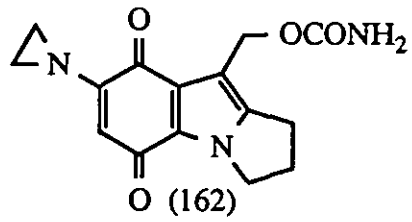
7-(Aziridin-1-yl)-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (160)
Distilled aziridine (1.0 cm^3) was added to a stirred solution of 7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (78) (0.030 g, 0.131 mmol) in anhydrous DMF (3.0 cm^3) under nitrogen. After stirring at room temperature for 15 h under nitrogen, water (5 cm^3) was added and extracted with dichloromethane (3 x 50 cm^3). The organic extracts were washed with water (5 x 50 cm^3), brine (75 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a red residue, which was purified by column chromatography (eluting with ethyl acetate) to give a red solid. Recrystallisation of the red residue from dichloromethane-light petroleum gave the title compound (160) (0.026 g, 84%) as a red solid, m.p. 163°C-165°C, (Found: M^+, 240.090. $C_{14}H_{12}N_2O_2$ requires M, 240.090); $\lambda_{max.}$ (MeOH) 237 (log ε 4.50), 308 (4.33), and 491 nm (3.34); $\nu_{max.}$ ($CHCl_3$) 1670, 1636, 1584, 1497, 1475, 1289, and 1261 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.29 (1 H, s, 9-H), 5.73 (1 H, s, 6-H), 4.26 (2 H, m, 3-CH_2), 2.34 (2 H, m, 1-H and 2-H), 2.18 (4 H, s, 2 x CH_2N [aziridine]), 1.28 (1 H, m, 1a-CHH), and 0.55 (1 H, m, 1a-CHH); δ_C (100.6 MHz; $CDCl_3$) 178.77 (CO, 8-C), 177.71 (CO, 5-C), 157.88 (C, 7-C), 146.39 (C, 4a-C), 128.05 (C, 9a/8a-C), 126.74 (C, 8a/9a-C), 115.92 (CH, 6-C), 98.96 (CH, 9-C), 49.73 (CH_2, 3-C), 27.60 (2 x CH_2N [aziridine]), 20.90 (CH, 1-C), 16.24 (CH_2, 1a-C), and 15.11 (CH, 2-C); m/z 241 (MH^+, 17%), and 240 (M^+, 100%).

Note: Assignment of 6-C and 9-C was achieved by using ^1H - ^{13}C Correlation Spectrum at 296 K.



(161)

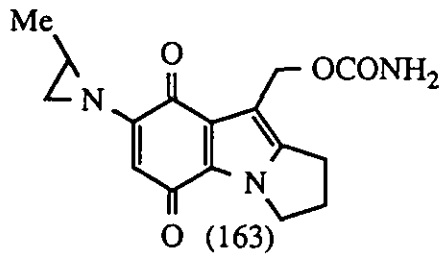
7-(2-Methylaziridin-1-yl)-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione. (161) Distilled 2-methylaziridine (1 cm^3) was added to a stirred solution of 7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (78) (0.030 g, 0.131 mmol) in anhydrous DMF (3.5 cm^3) under nitrogen. After stirring at room temperature for 15 h under nitrogen, more 2-methylaziridine (1 cm^3) was added and the reaction was monitored by TLC. After a further 48 h, water (5 cm^3) was added and extracted with dichloromethane (3 x 50 cm^3). The organic extracts were washed with water (5 x 50 cm^3), brine (75 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a red residue, which was purified by column chromatography (eluting with ethyl acetate) to give the title compound (161) (0.026 g, 79%) as a red oil, (Found: M^+, 254.1059 $C_{15}H_{14}N_2O_2$ requires M, 254.1055); $\lambda_{max.}$ (MeOH) 237 (log ε 4.38), 308 (4.20), and 493 nm (3.23); $\nu_{max.}$ ($CHCl_3$) 3013, 1668, 1634, 1581, 1497, 1288, 1259 and 1144 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.26 (1 H, s, 9-H), 5.68 (1 H, s, 6-H), 4.25 (2 H, s, 3-CH_2), 2.34 (2 H, m, 1-H and 2-H), 2.24 (1 H, m, N-C<u>H</u>Me), 2.07 (2 H, m, N-C<u>H</u>$_2$), 1.41 (3 H, d, J 5.5 Hz, N-CHC<u>H</u>$_3$), 1.27 (1 H, m, 1a-C<u>H</u>H), and 0.58 (1 H, m, 1a-CH<u>H</u>); δ_C (62.9 MHz; $CDCl_3$+TMS) 179.03 (CO, 8-C), 177.91 (CO, 5-C), 158.04 (C, 7-C), 146.37 (C, 4a-C), 128.08 (C, 9a/8a-C), 127.00 (C, 8a/9a-C), 115.25 (CH, 6-C), 98.93 (CH, 9-C), 49.82 (CH_2, 3-C), 36.15 (CH, N-<u>C</u>HMe), 34.53 (CH_2, N-<u>C</u>H_2), 21.05 (CH, 1-C), 17.64 (CH_3, Me), 16.35 (CH_2, 1a-C), 15.22 (CH, 2-C); m/z 255 (<u>MH</u>$^+$, 17%), and 254 (<u>M</u>$^+$, 100).



(162)

7-(Aziridin-1-yl)-9-hydroxymethyl-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione carbamate. (162)

Distilled aziridine (1 cm^3) was added to a stirred solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione carbamate (138) (0.030 g, 0.103 mmol) in anhydrous DMF (3.5 cm^3) under nitrogen. After stirring at room temperature for 72 h under nitrogen, water (5 cm^3) was added and extracted with dichloromethane (3 x 50 cm^3). The organic extracts were washed with water (5 x 50 cm^3), brine (75 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a red residue, which was recrystallised from dichloromethane-light petroleum to give the title compound (162) (0.021 g, 68%) as a red solid, m.p. 185°C (decomp.), $\lambda_{max.}$ (MeOH) 233 (log ε 4.24), 308 (4.13), and 483 nm (3.18); $\nu_{max.}$ (KBr disc) 3440, 3332, 1692, 1634, 1579, 1495, 1408, 1353, and 1260 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS+DMSO) 5.73 (1 H, s, 6-H), 5.52 (2 H, br s, NH_2), 5.21 (2 H, s, 10-CH_2), 4.20 (2 H, t, J 7.3 Hz, 3-CH_2), 2.93 (2 H, t, J 7.45 Hz, 1-CH_2), 2.53 (2 H, q [covered by DMSO peak], 2-CH_2), and 2.19 (4 H, s, 2 x CH_2 [aziridine]).

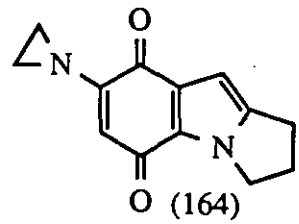


(163)

9-Hydroxymethyl-7-(2-methylaziridin-1-yl)-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione carbamate (163)

Distilled 2-methylaziridine (1 cm^3) was added to a stirred solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione carbamate (138) (0.030 g, 0.103 mmol) in anhydrous DMF (3.5 cm^3) under nitrogen. After stirring at room temperature for 72 h under nitrogen, water (5 cm^3) was added and extracted with dichloromethane (3 x 50 cm^3). The organic extracts were washed with water (5 x 50 cm^3), brine (75 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a red

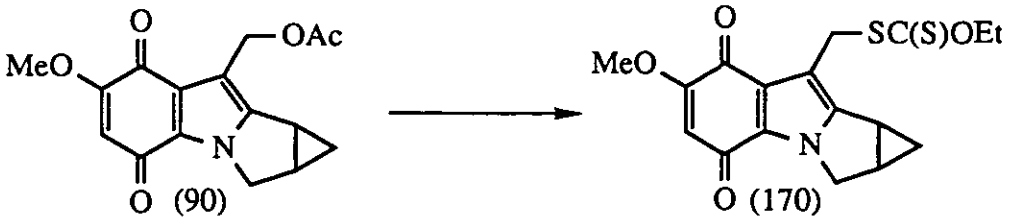
residue, which was recrystallised from dichloromethane-light petroleum to give the title compound (163) (0.019 g, 58%) as a red solid, m.p. 201°C (decomp.), (Found: M^+, 315.1227. $C_{16}H_{17}N_3O_4$ requires M, 315.1219); $\lambda_{max.}$ (MeOH) 232 (log ε 4.28), 308 (4.13), and 486 nm (3.21); $\nu_{max.}$ (KBr disc) 3342, 3201, 1725, 1663, 1622, 1576, 1497, 1262 and 1077 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS+DMSO) 5.72 (1 H, s, 6-H), 5.24 (2 H, s, 10-CH_2), 4.92 (2 H, br s, NH_2), 4.21 (2 H, t, J 7.3 Hz, 3-CH_2), 2.93 (2 H, t, J 7.4 Hz, 1-CH_2), 2.56 (2 H, q J 4.5 Hz, 2-CH_2), 2.27 (1 H, m, MeCHN [aziridine]), 2.08 (2 H, m, CH_2N [aziridine]), and 1.43 (3 H, d, J 5.5 Hz, Me); δ_C (100.6 MHz; $CDCl_3$+DMSO) 179.43 (CO, 8-C), 177.93 (CO, 5-C), 157.73 (C/CO, 7-C/urethane), 156.71 (CO/C, urethane/7-C), 144.01 (C, 4a-C), 126.67 (C, 8a/9a-C), 124.91 (C, 9a/8a-C), 114.98 (CH, 6-C), 111.70 (C, 9-C), 57.95 (CH_2, 10-C), 46.77 (CH_2, 3-C), 35.95 (CH, NCHMe), 34.34 (CH_2, N-CH_2), 27.00 (CH_2, 1-C), 22.94 (CH_2, 2-C), and 17.56 (CH_3, Me); m/z 315 (M^+, 28%), 254 (59), 228 (25), 44 (100).
Note: ^1H - ^{13}C Correlation spectrum was used to assign structure



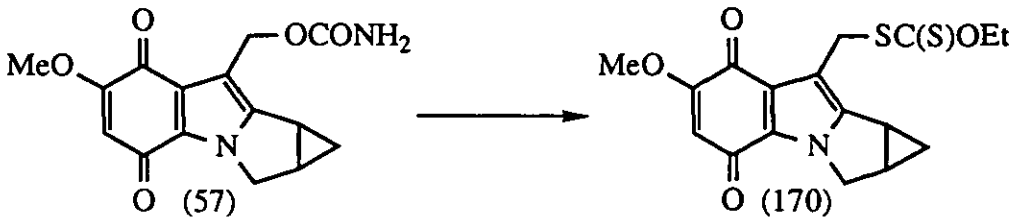
(164)

7-(Aziridin-1-yl)-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (164)
Distilled aziridine (1 cm^3) was added to a stirred solution of 7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (133) (0.030 g, 0.138 mmol) in anhydrous DMF (3.0 cm^3) under nitrogen. After stirring at room temperature under nitrogen for 15 h, water (5 cm^3) was added and extracted with dichloromethane (3 x 50 cm^3). The organic extracts were washed with water (5 x 50 cm^3), brine (75 cm^3), and dried ($MgSO_4$). Removal of solvent in vacuo gave a red residue, which was purified by column chromatography (eluting with ethyl acetate) to give a red solid. Recrystallisation of the red residue from dichloromethane-light petroleum gave the title compound (164) (0.023 g, 73%) as a red solid, m.p. 184-186°C, (Found: M^+, 228.0893. $C_{13}H_{12}N_2O_2$ requires M, 228.0899); $\lambda_{max.}$ (MeOH) 231 (log ε 4.34), 308 (4.22), and 479 nm (3.19); $\nu_{max.}$ ($CHCl_3$) 1670, 1638, 1583, 1475, and 1274 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.27 (1 H, s, 9-H), 5.75 (1 H, s, 6-H), 4.22 (2 H, t, J 7.2 Hz, 3-CH_2), 2.85 (2 H, t, J 7.4 Hz, 1-CH_2), 2.56 (2 H, q, J 7.3 Hz, 2-CH_2), and 2.19 (4 H, s, 2 x CH_2N [aziridine]); δ_C (62.9 MHz; $CDCl_3$+TMS) 179.11 (CO, 8-C), 178.06 (CO, 5-C), 157.96 (C, 7-C), 144.55 (C, 4a-C), 130.90 (C, 9a/8a-C), 128.82 (C, 8a/9a-C), 116.20 (CH, 6-C), 99.84 (CH,

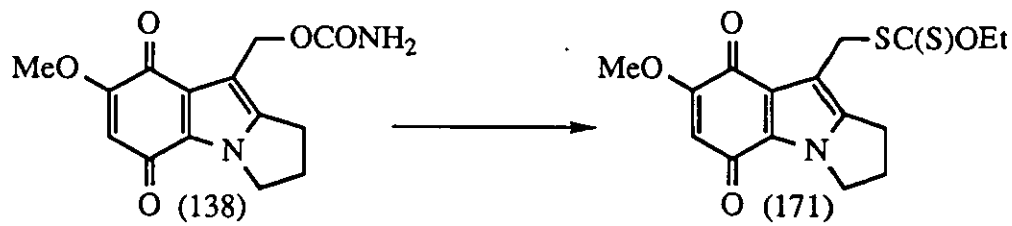
6.7. *Experimental For Chapter 4*



9-[(Ethoxythionothio)methyl]-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione. (170) A solution of 9-acetoxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (90) (0.020 g, 0.066 mmol) in a mixture of dichloromethane / methanol / water = 1:1:1 (20 cm^3) was purged with nitrogen (10 min) at room temperature. Subsequently, potassium ethyl xanthate (0.053 g, 0.332 mmol) was added followed by a solution of sodium dithionite (0.023 g, 0.132 mmol) in water (0.4 cm^3). After the mixture was stirred under nitrogen for 12 min, the reaction was complete and air was rapidly blown through the mixture. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (3 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a red residue, which was purified by column chromatography to give a red solid. Recrystallisation of the solid residue from dichloromethane-light petroleum gave the title compound (170) (0.018 g, 73%) as a red solid, m.p. 162-164°C, (Found: C, 56.1; H, 4.6; N, 3.8. $C_{17}H_{17}NO_4S_2$ requires C, 56.2; H, 4.7; N, 3.85%); $\lambda_{max.}$ (MeOH) 230 (log ε 4.26), 284 (4.32), 348 (3.48) and 474 nm (3.23); $\nu_{max.}$ ($CHCl_3$) 1671, 1637, 1596, 1500, 1229, and 1051 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.58 (1 H, s, 6-H), 4.66 (2 H, q, J 7.1 Hz, $C\underline{H}_2Me$), 4.58 (2 H, s, 10-CH_2), 4.26 (2 H, m, 3-CH_2), 3.79 (3 H, s, OMe), 2.55 (1 H, m, 1-H), 2.36 (1 H, m, 2-H), 1.41 (3 H, t, J 7.1 Hz, $CH_2C\underline{H}_3$), 1.32 (1 H, m,1a-C<u>H</u>H), and 0.55 (1 H, m, 1a-CH<u>H</u>); δ_C (62.9 MHz; $CDCl_3$+TMS) 214.99 (C, CS), 177.63 (CO, 8-C), 175.51 (CO, 5-C), 160.61 (C, 7-C), 145.66 (C, 4a-C), 111.35 (C), 105.57 (CH, 6-C), 69.98 (CH_2, C(S)O$\underline{C}H_2$Me), 56.50 (CH_3, OMe), 50.12 (CH_2, 3-C), 30.91(CH_2, CH_2S), 20.91 (CH, 1-C), 16.50 (CH_2, 1a-C), 15.15 (CH, 2-C), and 13.85 (CH_3, $C(S)OCH_2CH_3$).



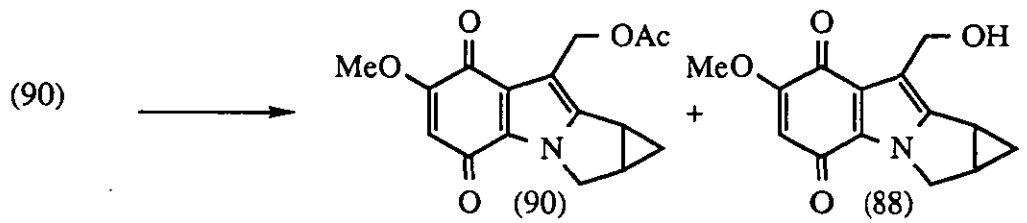
9-[(Ethoxythionothio)methyl]-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione. (170) A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57) (0.020 g, 0.066 mmol) in a mixture of dichloromethane / methanol / water = 1:1:1 (20 cm^3) was purged with nitrogen (10 min) at room temperature. Subsequently, potassium ethyl xanthate (0.053 g, 0.332 mmol) was added followed by a solution of sodium dithionite (0.023 g, 0.132 mmol) in water (0.4 cm^3). After the mixture was stirred under nitrogen for 10 min, the reaction was complete and air was rapidly blown through the mixture. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (3 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a red residue, which was purified by column chromatography to give a red solid. Recrystallisation of the solid residue from dichloromethane-light petroleum gave the title compound (170) (0.011 g, 46%) as a red solid. The spectroscopic data is the same as above.



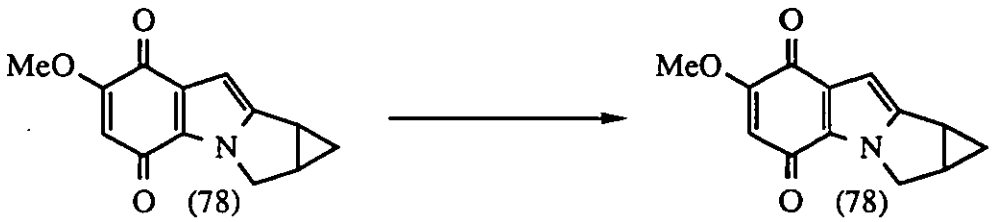
9-[Ethoxythionothio)methyl]-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione (171)

A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-pyrrolo[1,2-a]indole-5,8-dione carbamate (138) (0.030 g, 0.103 mmol) in a mixture of dichloromethane / methanol / water = 1:1:1 (30.6 cm^3) was purged with nitrogen (10 min) at room temperature. Subsequently, potassium ethyl xanthate (0.083 g, 0.518 mmol) was added followed by a solution of sodium dithionite (0.036 g, 0.206 mmol) in water (0.6 cm^3). After the mixture was stirred under nitrogen for 12 min, the reaction was complete and air was rapidly blown through the mixture. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (3 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in

vacuo gave an orange residue, which was purified by column chromatography to give an orange solid. Recrystallisation of the solid residue from dichloromethane-light petroleum gave the title compound (171) (0.015 g, 42%) as an orange solid, m.p. 142-143°C, (Found: M^+, 351.0600. $C_{16}H_{17}NO_4S_2$ requires M, 351.0600); $\lambda_{max.}$ (MeOH) (qualitative) 228, 282, 348 and 459 nm; $\nu_{max.}$ ($CHCl_3$) 1670, 1641, 1596, 1498, 1480, 1229, and 1051 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.60 (1 H, s, 6-H), 4.65 (2 H, q, J 7.1 Hz, CH_2Me), 4.55 (2 H, s, 10-CH_2), 4.22 (2 H, m, 3-CH_2), 3.80 (3 H, s, OMe), 2.94 (2 H, t, J 7.45 Hz, 1-CH_2), 2.56 (2 H, q, J 7.4 Hz, 2-CH_2), and 1.40 (3 H, t, J 7.1 Hz, $SC(S)OCH_2CH_3$); δ_C (62.9 MHz; $CDCl_3$+TMS) 215.14 (C, CS), 177.82 (C, CO), 160.59 (C, 7-C), 143.70 (C, 4a-C), 126.50 (C, 9a/8a-C), 124.40 (C, 8a/9a-C), 112.02 (C, 9-C), 105.72 (CH, 6-C), 70.06 (CH_2, $C(S)OCH_2Me$), 56.50 (CH_3, OMe), 47.08 (CH_2, 3-C), 30.96 (CH_2, CH_2S), 27.28 (CH_2, 1-C), 23.52 (CH_2, 2-C), and 13.85 (CH_3, $C(S)OCH_2CH_3$); m/z 351 (M^+, 7%), 230 (100), and 51 (29).

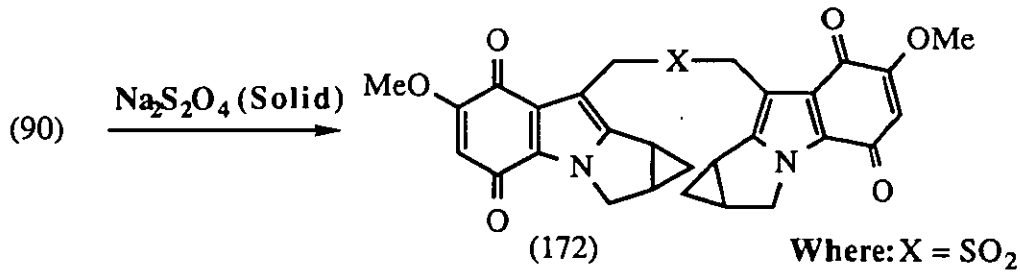


9-Acetoxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione. (90) A solution of 9-acetoxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (90) (0.010 g, 0.033 mmol) in a mixture of dichloromethane / methanol / water = 1:1:1 (10 cm^3) was purged with nitrogen (10 min) at room temperature. Subsequently, potassium ethyl xanthate (0.027 g, 0.168 mmol) was added (*No sodium dithionite was added in this experiment*). After the mixture was stirred under nitrogen for **6 h**, the reaction was complete and air was rapidly blown through the mixture. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (3 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography to give two solid, unreacted starting material (90) (0.008 g, 80%), and alcohol (88) (0.0015 g, 17%). The spectroscopic data for these compounds have previously been discussed.



Blank Experiment

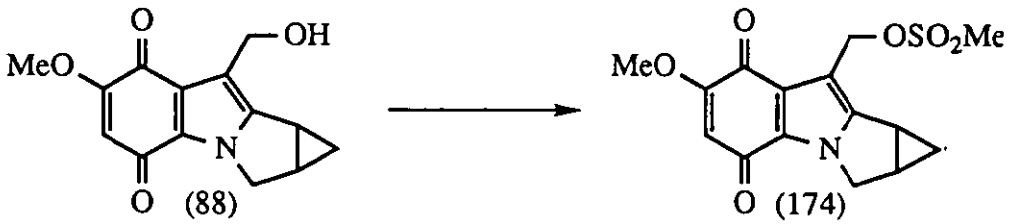
A solution of 7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (78) (0.020 g, 0.087 mmol) in a mixture of dichloromethane / methanol / water = 1:1:1 (26 cm^3) was purged with nitrogen (10 min) at room temperature. Subsequently, potassium ethyl xanthate (0.070 g, 0.437 mmol) was added followed by a solution of sodium dithionite (0.030 g, 0.172 mmol) in water (0.4 cm^3). After the mixture was stirred under nitrogen for 10 min, the reaction was complete and air was rapidly blown through the mixture. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (3 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography to give the starting material (78) (0.020 g, 100%) as an orange solid.



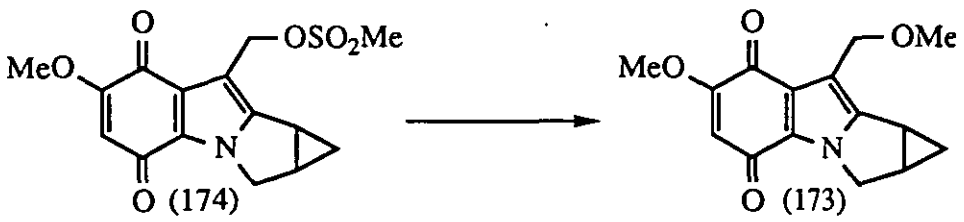
Dimer (172)

A solution of 9-acetoxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (90) (0.010 g, 0.033 mmol) in a mixture of dichloromethane / methanol / water = 1:1:1 (10 cm^3) was purged with nitrogen (10 min) at room temperature. Subsequently, excess methanol was added followed by solid sodium dithionite (0.012 g, 0.066 mmol). After the mixture was stirred under nitrogen for 10 min, the reaction was complete and air was rapidly blown through the mixture. The mixture was transferred to a separating funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (3 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave an orange residue, which was purified by column chromatography to give an orange solid. Recrystallisation of the

solid residue from dichloromethane-light petroleum gave the title compound (172) (0.005 g, 28%) as an orange solid, m.p. 238°C (decomp.), (Found: MH^+, 549.1355. $C_{28}H_{25}N_2O_8S$ requires MH, 549.1332); $\nu_{max.}$ ($CHCl_3$) 2845, 1671, 1641, 1597, 1114, and 1061 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 5.52 (1 H, s, 6-H), 4.77 (1 H, dd, J 1.6, 14.2 Hz, 10-CHH), 4.52 (1 H, dd, J 6.3, 14.3 Hz, 10-CHH), 4.28 (2 H, m, 3-CH_2), 3.72 (3 H, s, OMe), 2.65 (1 H, m, 1-H), 2.39 (1 H, m, 2-H), 1.28 (1 H, m,1a-CHH), and 0.72 (1 H, m, 1a-CHH); δ_C (100.6 MHz; $CDCl_3$) 177.13 (CO, 8-C), 177.04 (CO, 8-C), 176.92 (CO, 5-C), 160.19 (C, 7-C), 148.30 (C, 4a-C), 148.22 (C, 4a-C), 126.14 (C, 9a/8a-C), 126.10 (C, 9a/8a-C), 124.22 (C, 8a/9a-C), 105.60 (CH, 6-C), 101.34 (C, 9-C), 56.40 (CH_3, OMe), 50.34 (CH_2, 3-C), 49.32 (CH_2, 10-C), 49.23 (CH_2, 10-C), 21.04 (CH, 1-C), 16.20 (CH_2, 1a-C), 16.18 (CH_2, 1a-C), 15.18 (CH, 2-C), and 15.10 (CH, 2-C); m/z 571 (MNa^+, 58%), and 549 (MH^+, 100).

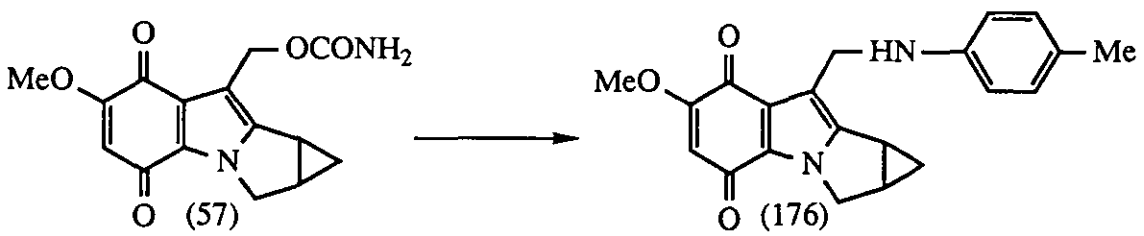


9-Methanesulphonyloxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione. (174) A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (88) (0.010 g, 0.039 mmol), triethylamine (0.007 cm^3, 0.047 mmol), in dry dichloromethane (2 cm^3) were stirred under nitrogen for 5 min at 0°C. Methanesulphonyl chloride (0.005 g, 0.047 mmol) was added, and the mixture stirred at room temperture for 15 h. After this time, water (2 cm^3), was added, and the mixture was extracted with dichloromethane (3 x 25 cm^3). The combined extracts were washed with water (2 x 25 cm^3), brine (25 cm^3), and dried ($MgSO_4$). The solvent was evaporated to give the title compound (174) (0.009 g, 69%), as a gummy solid, unstable to chromatography and used without further purification, $\nu_{max.}$ ($CHCl_3$) 1670, 1636, 1594, 1498, 1474, 1330, 1176, and 1062 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.60 (1 H, s, 6-H), 4.89 (2 H, m, 10-CH_2), 4.30 (2 H, m, 3-CH_2), 3.80 (3 H, s, OMe), 3.15 (3 H, s, Me), 2.49 (1 H, m, 1-H), 2.39 (1 H, m, 2-H), 1.30 (1 H, m ,1a-CHH), and 0.62 (1 H, m, 1a-CHH).



9-Methoxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (173)

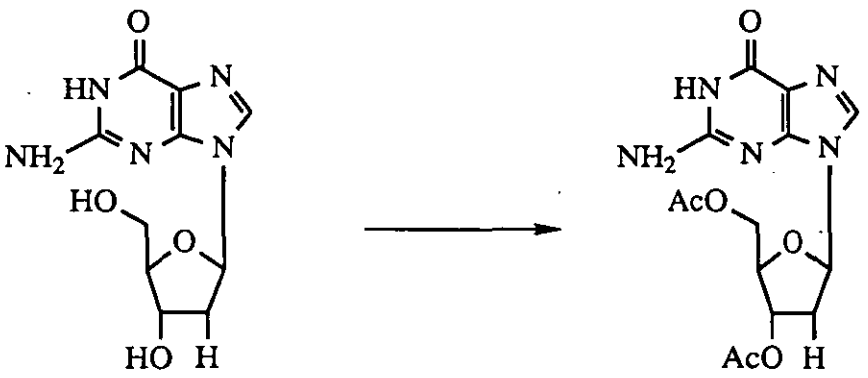
Sodium metal (0.0006 g, 0.027 mmol) in dry methanol (4.0 cm^3) was added to 9-methanesulphonyloxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione (174) (0.009 g, 0.027 mmol). The mixture was stirred at room temperture under nitrogen. After 2 h, water (3 cm^3) was added, and the mixture was extracted with ethyl acetate (3 x 25 cm^3). The combined extracts were washed with water (2 x 20 cm^3), brine (25 cm^3), dried ($MgSO_4$), and evaporated. Recrystallisation of the residue from dichloromethane-light petroleum gave the title compound (173) (0.005 g, 71%) as an orange solid, m.p. 148-150°C (lit.[79] 103-105°C), (Found: M^+, 273.0989. $C_{15}H_{15}NO_4$ requires M, 273.1001); $\nu_{max.}$ ($CHCl_3$) 1668, 1634, 1594, 1494, 1264 and 1136 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 5.58 (1 H, s, 6-H), 4.66 (2 H, m, 10-CH_2), 4.26 (2 H, m, 3-CH_2), 3.79 (3 H, s, 7-OMe), 3.45 (3 H, s, OMe), 2.45 (1 H, m, 1-H), 2.33 (1 H, m, 2-H), 1.30 (1 H, m, 1a-CHH), and 0.57 (1 H, m, 1a CHH); m/z 275 (MH_2^+, 5%), 274 (MH^+, 15), 273 (M^+, 84), and 272 (100).



9-(4-Methylphenyl)aminomethyl)-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo [1,2-a]indole-5,8-dione (176)

A solution of 9-hydroxymethyl-7-methoxy-1,2-dihydro-3H-1,2-cyclopropapyrrolo[1,2-a]indole-5,8-dione carbamate (57) (0.015 g, 0.050 mmol) in a mixture of dichloromethane / methanol / water = 1:1:1 (14.7 cm^3) was purged with nitrogen (10 min) at room temperature. Subsequently, *p*-toluidine (0.027 g, 0.250 mmol) was added followed by a solution of sodium dithionite (0.017 g, 0.100 mmol) in water (0.3 cm^3). After the mixture was stirred under nitrogen for 12 min, the reaction was complete and air was rapidly blown through the mixture. The mixture was transferred to a separating

funnel, and extracted with dichloromethane (3 x 30 cm^3). The combined extracts were washed with water (3 x 25 cm^3), brine (30 cm^3), and dried ($MgSO_4$). Removal of the solvent in vacuo gave a red residue, which was purified by column chromatography to give a red oil. Recrystallisation of the red residue from dichloromethane-light petroleum gave the title compound (176) (0.009 g, 53%) as a red solid, m.p. 71-73°C, (Found: M^+, 348.1474. $C_{21}H_{20}N_2O_3$ requires M, 348.1475); $\nu_{max.}$ ($CHCl_3$) 3020, 2928, 1667, 1638, 1596, 1519, 1496, 1232, and 1138 cm^{-1}; δ_H (250 MHz; $CDCl_3$+TMS) 6.94 (2 H, d, J 8.2 Hz, AA'BB'), 6.60 (2 H, d, J 8.4 Hz, AA'BB'), 5.56 (1 H, s, 6-H), 4.38 (2 H, s, 10-CH_2), 4.23 (2 H, m, 3-CH_2), 3.80 (3 H, s, OMe), 2.38 (2 H, m, 1-H and 2-H), 2.20 (3 H, s, Me), 1.32 (1 H, m,1a-CHH), and 0.53 (1 H, m, 1a-CHH); δ_C (62.9 MHz; $CDCl_3$+TMS) 177.80 (CO, 8-C), 177.68 (CO, 5-C), 160.60 (C, 7-C), 145.59 (C), 144.88 (C), 129.56 (CH, 2 x CHN-Ar), 126.78 (C), 113.88 (CH, 2 x CH-Ar), 105.56 (CH, 6-C), 56.55 (CH_3, OMe), 49.90 (CH_2, 3-C), 39.18 (CH_2, 10-C), 20.91 (CH/CH_3, 1-C/Me), 20.40 (CH_3/CH, C-1/Me), 16.36 (CH_2, 1a-C), and 14.55 (CH, 2-C); m/z 349 (MH^+, 23%), 348 (M^+, 94), 242 (100), and 106 (45).



(177) (178)

2'-Deoxy-3',5'-di-*O*-acetyl guanosine (178)

Acetic anhydride (0.18 cm^3, 1.873 mmol) was added to a stirred suspension of dry 2'-deoxyguanosine (177) (0.200 g, 0.749 mmol), 4-dimethylaminopyridine (0.015 g, 0.123 mmol) in a mixture of dry acetonitrile (10.00 cm^3) and triethylamine (0.27 cm^3, 1.947 mmol). After 2 h, methanol (5 cm^3) was added, and stirring was continued for 15 min. The reaction mixture was then filtered to give a colourless solid residue. The residue was purified by washing with ethanol and diethyl ether to give the title compound (178) (0.221 g, 84%) as a colourless solid, m.p. 222°C (decomp.) (lit.[104] 222°C [decomp.]), δ_H (250 MHz; $CDCl_3$+TMS+DMSO) 10.68 (1 H, s, NH), 7.78 (1 H, s, NCH=N), 6.30 (2 H, s, NH_2), 6.29 (1 H, m, 1'-H), 5.40 (1 H, m, 4'-H), 4.30 (3 H, m, 3'-H and 5'-CH_2), 2.92 (1 H, m, 2'-CHH), 2.47 (1 H, m, 2'-CHH), 2.10 (3 H, s,

Me), and 2.07 (3 H, s, Me); δ_C (62.9 MHz; $CDCl_3$+TMS+DMSO) 107.07 (C, OC(O)Me), 169.92 (C, OC(O)Me), 156.64 (C), 153.68 (C), 151.01 (C), 135.06 (CH, NCH=N), 116.69 (C), 82.49 (CH, sugar), 81.37 (CH, sugar), 74.39 (CH, sugar), 63.54 (CH_2, 5'-C), 35.36 (CH_2, 2'-C), 20.72 (CH_3, Me), and 20.47 (CH_3, Me).

6.8. Experimental for Chapter 5

Electrochemical Measurements in a Non-Aqueous Solvent

Sets of cyclic voltammograms at a sequence of scan rates (ranging from 20 mVs^{-1} to 200 mVs^{-1}) were obtained in triplicate using an E.G. & G. Princeton Applied Research Model 273 Potentiostat with a B.B.C. Goerz Metrawatt SE 790 Chart Recorder. A three-electrode system was employed with a platinum flag working electrode (1 cm^2). The platinum working electrode was pretreated by anodisation, then cathodisation, for 5 min each in sulphuric acid (0.5 M) at 100 mA, then washed thoroughly with de-ionised water and dried. The reference electrode was a sodium chloride saturated calomel electrode (s.s.c.e.) with a platinum-mesh counter electrode. Mitomycin C and analogues were prepared as 1 mM solutions in freshly distilled DMF (10.0 cm^3) containing dried tetrabutylammonium tetrafluoroborate (0.1 M) as the supporting electrolyte. Measurements were conducted at 22 (±1)°C in solutions freed of oxygen by bubbling with solvent-saturated nitrogen. Ferrocene was used as an internal reference.

Formal redox potential, E^f, for the one-electron reduction of the quinone moieties to the radical anion were calculated as follows:

$$E^f = 0.5\ (E_{p.c} + E_{p.a})$$

$E_{p.c}$ = Cathodic peak potential

$E_{p.a}$ = Anodic peak potential

Volume of cell = 10 cm^3

Electrochemical Measurements in an Aqueous Solvent

Sets of cyclic voltammograms at a sequence of scan rates (ranging from 50 mVs^{-1} to 500 mVs^{-1}) were obtained using an E.G. & G. Princeton Applied Research Model 273 Potentiostat with a B.B.C. Goerz Metrawatt SE 790 Chart Recorder. A three-electrode system was employed with a hanging drop mercury (Metrohm) working electrode. The reference electrode was a sodium chloride saturated calomel electrode (s.s.c.e.) with a platinum-mesh counter electrode. Mitomycin C and analogues were prepared as 0.5 mM

solutions in a mixture of freshly distilled DMF (1.0 cm^3) and a phosphate buffer at 7.4 (9.0 cm^3). Measurements were conducted at 22 (±1)°C in solutions freed of oxygen by bubbling with solvent-saturated nitrogen.

$E_{p.c}$ = Cathodic peak potential
Volume of cell = 10 cm^3

Note: All the biological assays were carried out under the direction of Dr Ian Stratford, MRC Radiobiology Unit, Didcot.

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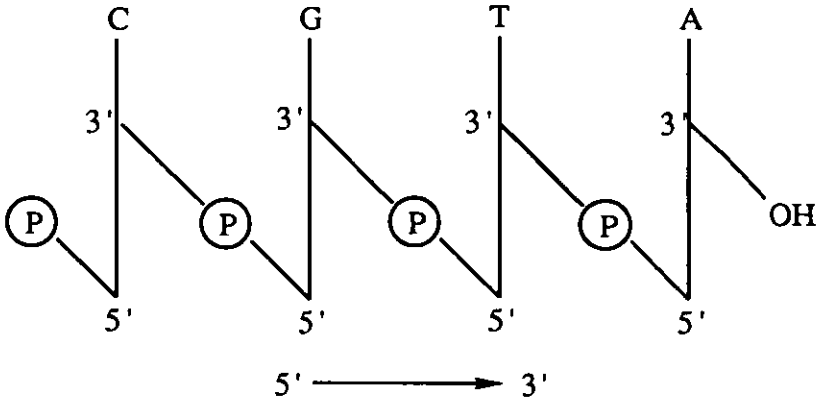
APPENDIX

Appendix 1

DNA (deoxyribonucleic acid): A polynucleotide having a specific sequence of deoxyribonucleotide units and serving as the carrier of genetic information.
Deoxyribonucleotide: A nucleoside phosphorylated at the 5' end of its pentose, 2'-deoxyribose.
Deoxyribonucleoside: A compound consisting of a purine or pyrimidine base covalently linked to a pentose, 2'-deoxyribose.
DNA contain two principal pyrimidine bases, cytosine (C) and thymine (T), and two principal purine bases, adenine (A) and guanine (G). The recurring deoxyribonucleotide units of DNA contain 2'-deoxy-D-ribose. The successive nuceotides of DNA are covalently linked to each other through phosphate-group "bridges." The 5'-hydroxy group of the pentose of one nucleotide unit is joined to the 3'-hydroxy group of the pentose of the next nucleotide by a phosphodiester linkage.

The deoxyribonucleotides units of DNA (deoxyribonucleosides 5'-monophosphates) are usually symbolised as A, G, T, and C (or less often as dA, dG, dT, and dC). In the free form at pH 7.0 these nucleotides are commonly abbreviated dAMP, dGMP, dTMP, and dCMP, *i.e.* Deoxyguanylate, deoxyguanosine 5'-monophosphate, symbols: G, dAGP.

The nucleotide sequences of nucleic acids can be represented schematically, as illustrated by a segment of DNA having four nucleotide units.

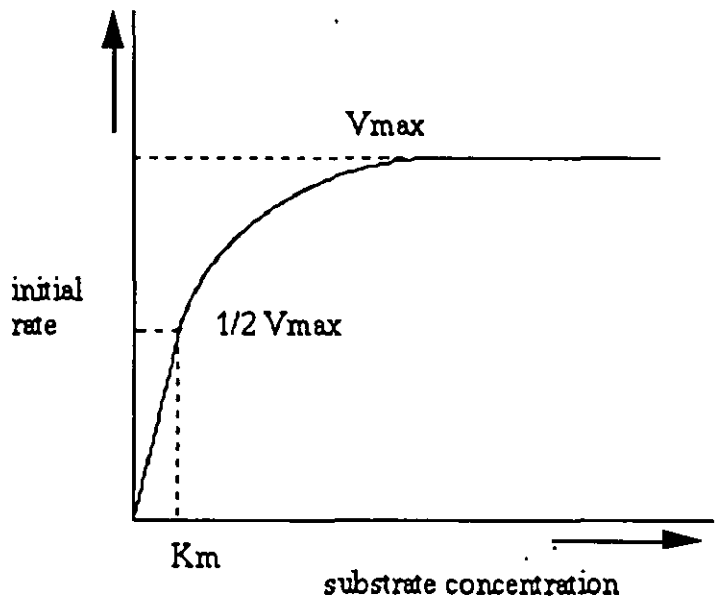


The bases are symbolised by A, T, G, and C, each deoxyribose by vertical line, and the phosphate groups by P. The numbers give the position in the deoxyribose units to which the phoshate groups are attached. The structure of a single strand of DNA is always written with the 5' end at the left and the 3' end at the right. Three simpler representations of the above deoxyribonuceotide are illustrated below.
pCpGpTpA / pCGTA / CGTA

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

Where: E = Enzyme
S = Substrate
P = Product

There is a quantitative relationship between the substrate concentration and the rate of an enzymatic reaction which was derived by Michaelis and Menten. Many enyzmes show the typical hyperbolic curve, as illustrated below relating reaction velocity to substrate concentration, with a gradual approach to saturation of the enzyme with substrate. The Michaelis-Menten equation is an algebrias expression of the hyperbolic shapes of such curves, in which the important terms are substrate concentration [s], initial velocity [v_o], V_{max} (maximum velocity towards which the rate approaches at infinite substrate concentration, and K_M (substrate concentration giving half the maximal velocity). This equation as illustrated below and is fundamental to all studies of enzyme kinetics, in which K_M and V_{max} are constants of an enzyme for a particular substrate. A good enzyme will have a high V_{max} and low K_M value.



Michaelis-Menten Equation: An equation relating velocity and the substrate concentration of an enzyme.

$$v_o = \frac{V_{max}[s]}{K_M + [s]}$$

Where: v_o = Initial rate at substrate concentration [s]
V_{max} = Maximum rate
K_M = Michaelis - Menten constant of an enzyme for a particular substrate