

SUBSTITUTED MITOSENES:
SYNTHESIS AND ANTINEOPLASTIC ACTIVITY

by

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In memory of my father, John Hodges, V, who upon reading this dissertation most assuredly would have said, "For God's sake don't be all Ph.D. and no common sense!"

PREFACE

The status of cancer as the second most common cause of death in the United States is a fact of life that in many ways is a result of the technologically advanced society we live in. As medical science has enabled us to escape many fatal illnesses, more and more of us will face death by a group of yet unconquered diseases known as cancer. While technology has improved our lives with rapid transportation, computerized communication and advanced methods for food production, the by-products of technology are seeping into the earth and our bodies, ever increasing the risk of cancer for the population of tomorrow. It is impossible and undesirable to turn back the wheels of technology so we are left with the task of directing technology to solve the problems it has created. The elimination of cancer will probably not happen until man, through his technology, finds the way to prevent its occurrence. For the time being it is also necessary to direct our efforts toward treatment and attempted cures for the cancers that are inevitable. The search for new chemotherapeutic agents is only one facet of the overall effort to eradicate cancer and the following account of research is only a small fragment of the work in this field.

The purpose of this study was to create synthetic analogs of the naturally occurring antineoplastic drug, mitomycin C. The clinical utility of mitomycin C is limited to a great extent by its toxicity so it was hoped that through the appropriate modifications in chemical structure a more attractive agent could be found. The direction taken in this study was to search for compounds with significant antineoplastic activity followed by analysis of their toxicity. Specifically, a series of mitosene analogs were prepared by total synthesis and their anti-tumor activity screened against an animal tumor model, P388 murine leukemia. Analysis of the test data indicated that certain analogs exceeded minimal activity standards but unfortunately none were sufficiently active in this initial screen when compared to other mitomycin analogs to warrant further study of toxicity. Hence the only hope for the compounds prepared ever becoming useful drugs lies in as yet uncompleted tests in other antitumor assays which could show activity against other types of cancer. Hopefully the seemingly negative results of this study will help direct others to more positive achievements with this class of antineoplastic agents.

The author wishes to express an immense gratitude to Dr. William A. Remers for his priceless contributions of guidance, patience and support as a dissertation director. In addition, special thanks go to Dr. William T. Bradner of

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TABLE OF CONTENTS

	Page
LIST OF ILLUSTRATIONS	x
LIST OF TABLES	xi
ABSTRACT	xii
INTRODUCTION	1
Structure	1
Pharmacology	3
Molecular Mode of Action	4
Biosynthesis	10
Structure-Activity Relationships	11
CHEMISTRY	15
Target Compounds	15
Synthesis of Substituted Mitosenes	16
Mass Spectral Fragmentation	31
Mitosane vs. Mitosene	38
Acid Reactions	38
Base Reactions	40
Reduction	42
ANTINEOPLASTIC ACTIVITY AND DISCUSSION	48
Activity Screening	48
Screen Results	50
Discussion	53
EXPERIMENTAL	60
General	60
2,5-Dimethyl-4-nitrophenol (2)	61
2,5-Dimethyl-4-nitroanisole (3)	62
5-Methoxy-4-methyl-2-nitrophenyl- pyruvic Acid (4)	63
5-Methoxy-6-methyl-2-indolecarboxylic Acid (5)	64
Methyl 5-methoxy-6-methyl-2-indole- carboxylate (6)	65

TABLE OF CONTENTS--Continued

	Page
Methyl 2,3-dihydro-7-methoxy-6-methyl-1-oxo-1H-pyrrolo[1,2-a]indole-2-carboxylate (7)	66
2,3-Dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-1-one (8)	67
2,3-Dihydro-1-hydroxy-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole (9)	67
1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole (10)	68
1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde (11)	69
1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde (12)	70
1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-5,8-dioxo-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde (14)	71
1-Acetoxy-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-5,8-dione (15)	72
1-Acetoxy-7-methoxymitosene (17)	75
1-Hydroxy-7-methoxymitosene (19) and 7-Methoxymitosene (18)	76
1-Hydroxy-7-methoxymitosene Carbamate	77
1-Hydroxy-7-methoxymitosene Methylcarbamate (21)	79
1-Hydroxy-7-methoxymitosene Methylcarbonate (22)	80
1-Hydroxy-7-methoxymitosene Trimethylacetate (23)	81
1-Hydroxy-7-methoxymitosene Chloroacetate (24)	82
1-Hydroxy-7-methoxymitosene Nicotinate (25)	82
7-(1-Aziridinyl)-1-hydroxymitosene Acetate (26)	83
7-(1-Aziridinyl)-1-hydroxymitosene Carbamate (27)	84
9-Chloromethyl-2,3-dihydro-1-hydroxy-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-5,8-dione Methylcarbamate (36)	86
2,3-Dihydro-1-hydroxy-9-hydroxymethyl-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole Diacetate (29)	87

TABLE OF CONTENTS--Continued

	Page
2,3-Dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-5,8-dione-1-oxime Carbamate (7-Methoxymitosene-1-oxime, <u>32</u>)	88
Comparative Acid Hydrolysis of Mitosenes.	90
Acid Hydrolysis of 1-Hydroxy-7-methoxy-mitosene Nicotinate (<u>25</u>)	91
APPENDIX A: MASS SPECTRAL DATA	92
REFERENCES	104

LIST OF ILLUSTRATIONS

	Page
Figure 1: Naturally occurring mitomycins	2
Figure 2: <u>Chemical Abstracts</u> numbering system.	3
Figure 3: Bioreductive alkylation.	6
Figure 4: Acid catalysed alkylation.	9
Figure 5: Biosynthetic precursors of porfiromycin.	10
Figure 6: Analogs of mitomycins.	12
Figure 7: Target compounds	16
Figure 8: Mitosene synthesis	21
Figure 9: Miscellaneous mitosenes.	30
Figure 10: Fragment structures for 1-Acetoxy-7-methoxymitosene	35
Figure 11: 7-Methoxy derivative fragmentation.	36
Figure 12: 7-(1-Aziridinyl) derivative fragmentation	37
Figure 13: Reactions of mitosanes and mitosenes.	44

LIST OF TABLES

	Page
Table 1: Activities of substituted mitosenes vs. P388 leukemia	51
Table 2: Activities of aziridinomitosenes vs. P388 leukemia	52

ABSTRACT

As analogs of the naturally occurring mitomycin anti-tumor antibiotics, a series of novel mitosenes were prepared by total synthesis and tested for antineoplastic activity. The synthetic route followed used literature-based methods to prepare 1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde from 2,5-Dimethylphenol. This intermediate was subsequently converted to 1-Acetoxy-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-5,8-dione using newly developed procedures that dramatically increased the yield compared to earlier techniques. Literature methods were then again followed to obtain 2,3-Dihydro-1-hydroxy-9-hydroxymethyl-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]-indole-5,8-dione Carbamate (7-Methoxy-1-hydroxymitosene) which was the major intermediate from which various 1-substituted and 1,7-disubstituted mitosenes were prepared. The analogs were screened for antineoplastic activity using the lymphocytic leukemia P388 assay according to National Cancer Institute protocol. Of thirteen mitosene analogs prepared nine were active with %T/C values ranging from 128 to 167 (mitomycin C T/C = 200). These compounds were quite potent with optimum dosage at 6.4 mg/kg for the majority of the active analogs (mitomycin C O.D. = 3.2 mg/kg). A high frequency

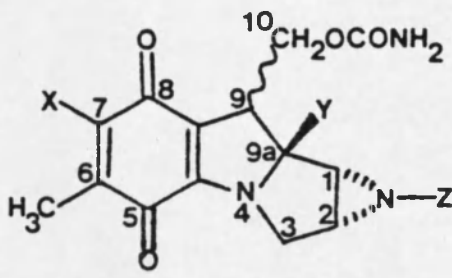
of toxic responses were seen at doses above 12.8 mg/kg. When compared to semisynthetic aziridinomitosenes the compounds prepared by total synthesis were less effective against P388 leukemia although in general they were more potent. The observed activity of this series of analogs supports the theory of bioreductive alkylation proposed for the mitomycins. In addition to the synthetic work and biological testing, mass spectral data were obtained and fragmentation patterns elucidated.

INTRODUCTION

Structure

A prerequisite for the full understanding of the chemical and medicinal properties of mitosenes is a thorough knowledge of their parent compounds, the mitomycins. During the late 1950's three closely related molecules with antibiotic and antineoplastic activity were isolated from the fermentation broth of several Streptomyces species by researchers in Japan¹⁻³. These products were named mitomycin A, B and C but their structures were undetermined at the time. Soon afterward a similar compound, porfiro-mycin was isolated in the United States⁴ which eventually led to the structure elucidation of all four compounds in the 1960's. One final native member of this family named mityromycin was also isolated in the U.S. during the early 1960's⁵. The structures of naturally occurring mitomycins and the numbering system to be used throughout this publication are given in Figure 1. This numbering system was chosen for simplicity in correlation of positions between mitomycins and the analogs of interest, mitosenes. It differs from that designated by Chemical Abstracts (Figure 2) for mitomycin C. Spectral and chemical evidence supporting the designated structures has been summarized by Remers¹. In addition an X-ray diffraction analysis of

mitomycin C has recently been published by Ogawa, et al.⁶
 The trivial name mitosane, where x, y and z = H is used to
 describe the basic tetracyclic unit. The term mitosene
 refers to the unit modified by the elimination of the ele-
 ments of methanol from the 9 and 9a-positions (see Figure
 6).



Compound	X	Y	Z	9-Substituent
Mitomycin A	CH ₃ O-	-OCH ₃	-H	α
Mitomycin B	CH ₃ O-	-OH	-CH ₃	β
Mitomycin C	H ₂ N-	-OCH ₃	-H	α
Porfiromycin	H ₂ N-	-OCH ₃	-CH ₃	α
Mitryomycin	CH ₃ O-	-NHCO- ¹⁰ C O	-CH ₃	α

Figure 1: Naturally occurring mitomycins.

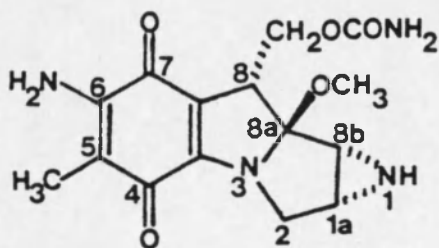


Figure 2: Chemical Abstracts numbering system.

Pharmacology^{1,7}

The toxicity of mitomycins is too severe to permit their use as general antibiotics despite their broad spectrum activity. For the purpose of cancer chemotherapy, however, the toxicity is considered tolerable when compared to other established agents. Mitomycin C is the only native mitomycin that has been approved to date for standard medical use as an antineoplastic drug in the United States and is marketed by Bristol Laboratories as Mutamycin^R. Porfiromycin, considerably less active but also less toxic, has attained investigational status for cancer chemotherapy.

Mitomycin C has not become one of the most popular chemotherapeutic agents in the U.S. because of its narrow therapeutic ratio. The drug has, however, received a great deal of enthusiasm in Japan where an estimated half of the

chemotherapy regimens included mitomycin C during the 1960's. This fact along with the development of improved dosage schedules and combination regimens such as FAM (5-fluorouracil, adriamycin, mitomycin C), MA (mitomycin C, adriamycin) and MOB (mitomycin C, oncovin, bleomycin) offer a good deal of promise for increased usage of mitomycin C. Indications include adenocarcinomas of the stomach, pancreas and colon, bladder carcinomas and squamous cell carcinoma of the cervix.

As an alkylating agent mitomycin C exhibits some of the toxicities traditionally associated with agents such as mechlorethamine. They include bone marrow depression resulting in leukopenia, thrombocytopenia, anemia and immunosuppression, nausea, vomiting and mutagenicity. By far the limiting acute toxicity is leukopenia. Thrombocytopenia and anemia are less prevalent. On a long term basis the mutagenicity of mitomycin C is of concern because of possible teratogenesis and induction of secondary neoplasms. It has the advantage of lessened irritation at the site of administration compared to traditional alkylating agents due to its apparent need for bioactivation.

Molecular Mode of Action

On a molecular basis the mode of action of mitomycins is proposed to involve initial bioactivation followed by alkylation of DNA and other essential cell macromolecules.

Subsequently the syntheses and functions of DNA, RNA, proteins, etc. are inhibited or eliminated. The overall effect is thus the disruption of normal cellular activity leading to a decreased mitotic rate or cell death. Summaries of evidence supporting the proposed mechanism of action for mitomycins have been compiled by Lown⁷ and Remers¹. In addition, two recent publications by Tomasz⁸ and Hornemann⁹ offer in vitro mechanistic evidence. The postulated process which is proposed for a number of natural antineoplastic substances¹⁰ is called bioreductive alkylation.

The functional groups involved in the activation and alkylation processes are the quinone carbonyls, the 9a-substituent, the 10-carbamate and the 1,2-aziridine. The molecular mode of action is summarized in Figure 3 using mitomycin C for illustrative purposes. Bioactivation begins with the reduction of the quinone I to the hydroquinone II by an enzymatically mediated process involving cellular reducing agents such as NADPH. This is followed by the elimination of the elements of methanol from the 9 and 9a-positions yielding an indolohydroquinone, III. The electron rich indole system, further activated by an amino and two hydroxyl groups, easily eliminates substituents from the 1 and 10-benzylic-like positions giving mitomycins the capability of being either monofunctional or bifunctional alkylating agents. In the case of bifunctional alkylation, opening of the aziridine ring as shown

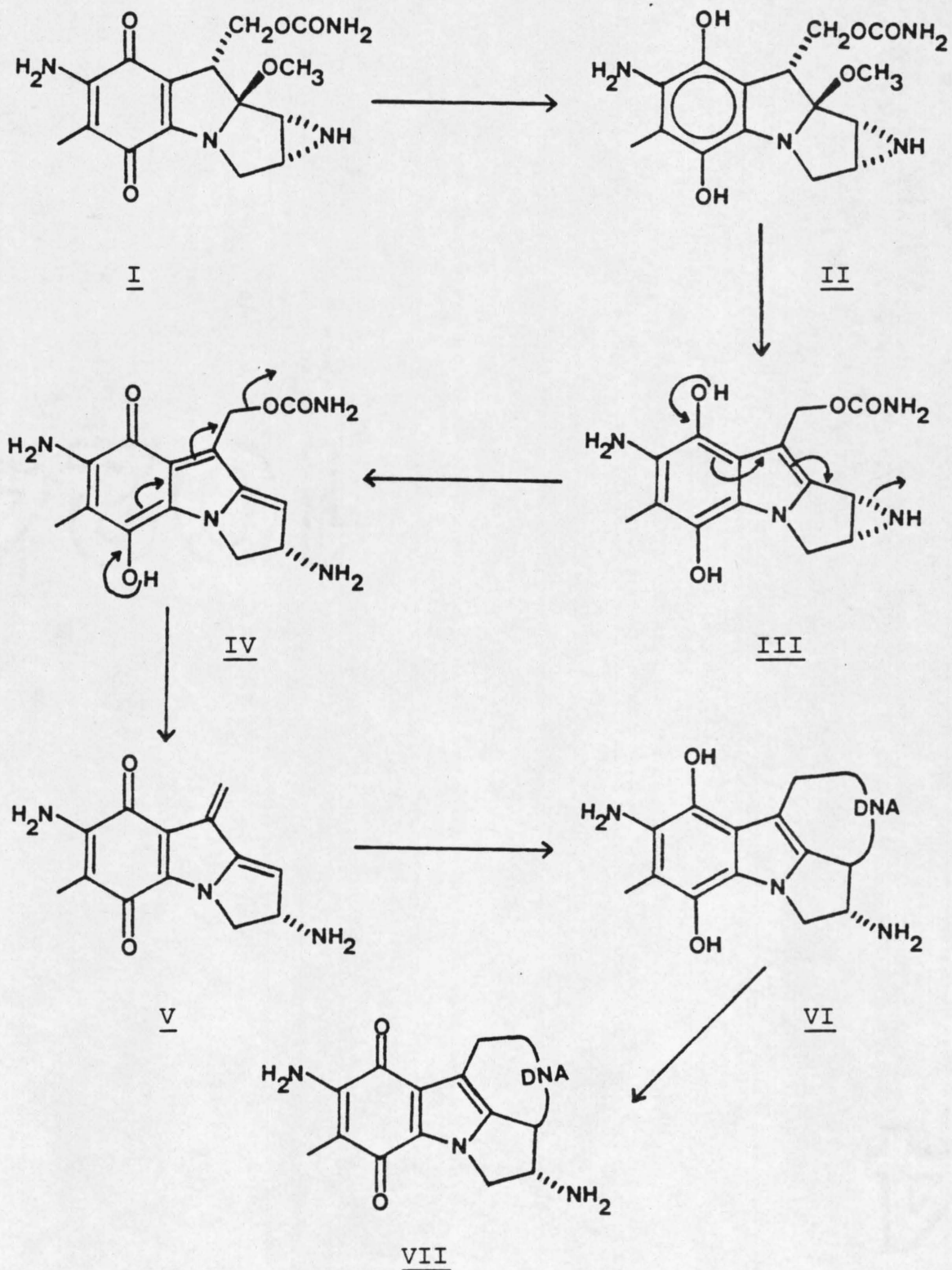


Figure 3: Bioreductive alkylation.

gives IV, containing a Michael acceptor in the 1-position. Elimination of the carbamate moiety from the 10-position yields a similar alkylating function to give V. Various nucleophilic groups on cellular macromolecules can then substitute in the reverse order from the eliminations to achieve bifunctional alkylation.

Spontaneous oxidation of the hydroquinone would afford a more permanent alkylation as in VII. This last step is associated with a second cause of cellular damage. The spontaneous oxidation of reduced mitomycins in vitro has been shown to liberate hydrogen peroxide¹¹, an agent known to cause cleavage of the phosphate-deoxyribose backbone of DNA molecules. Thus entities such as VI when oxidized in vivo may be responsible for degradation of DNA in addition to the deleterious effects normally associated with alkylation. Such a process has been demonstrated in the form of sister chromatid exchanges found in cell cultures of patients treated with mitomycin C^{12,13}. Cyclic reduction and oxidation of mitomycin molecules bound covalently to DNA would multiply the extent of DNA fragmentation.

Cross linking of the DNA double helix is considered to be the most lethal event in cancer cells since this prevents DNA replication¹⁴. This of course requires a bifunctional alkylating agent with sufficiently large separation between the alkylating functions to bridge the space from

one DNA strand to its complement. Mitomycin C has been shown to fit this requirement since chemical reduction in the presence of DNA has been shown to result in measurable cross alkylated DNA in addition to monofunctional alkylation^{1,7}.

It is important to note that monofunctional alkylation is possible without reductive activation (Figure 4). Under acidic conditions the 9a-methoxyl group readily eliminates to give the indoloquinone, VIII. This enhances the acid lability of the aziridine ring such that alkylation of nucleophiles by the 1-position is easily accomplished (XI). Tomasz⁸ has shown that such monofunctional alkylation is possible in physiological pH ranges but reports no alkylation at the 10-position under these conditions. Such acid catalysed monofunctional alkylations may be responsible for much of the alkylating toxicity of mitomycins. In addition one could imagine a combination of acid catalysed and bio-reductive mechanisms to give bifunctional alkylation. For instance an acid catalysed process could result in alkylation involving the 1-position on a DNA strand. The cell in an attempt to remove the foreign molecule might reduce the quinone, activating the 10-position for a second alkylation on the complementary DNA strand to give a cross link.

An overview of the proposed molecular actions of native mitomycins reveals the importance of mitosenes. In both bioreductive and acid catalysed mechanisms mitosenes

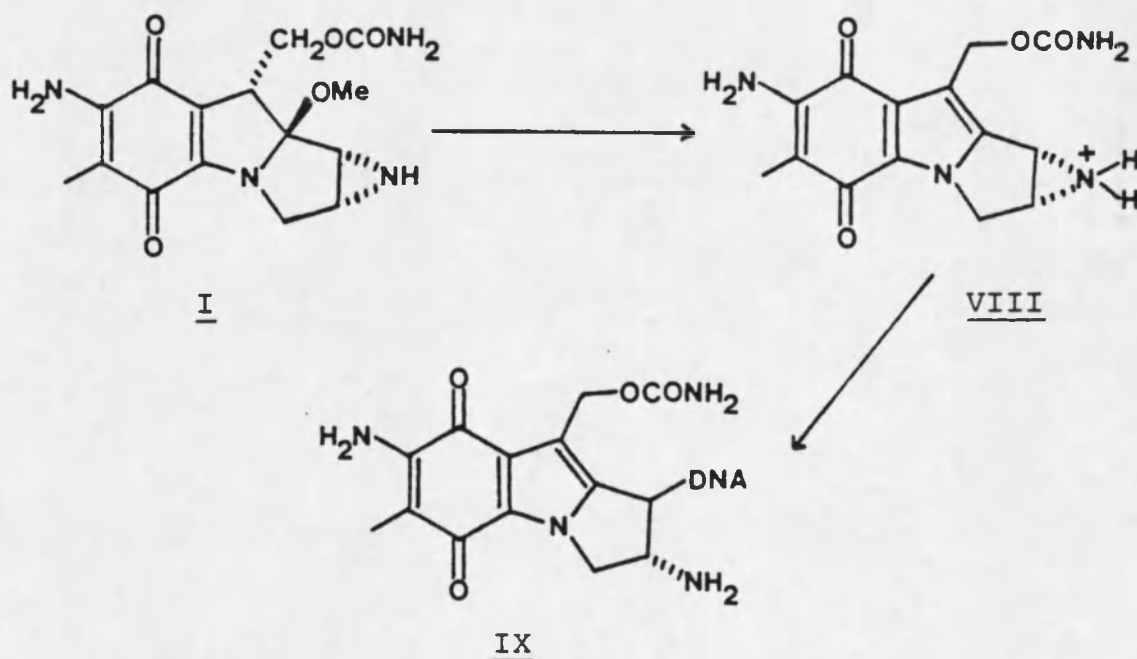


Figure 4: Acid catalysed alkylation.

are key intermediates in the alkylation process. By the bioreductive pathway one can consider mitosenes to be partially activated species. Quinone reduction is still required for activity but elimination of methanol is not. With acid catalysed, monofunctional alkylation mitosenes are the completely activated entities. The proper pH is all that is necessary for activity. Finally, the cyclic oxidation and reduction of mitosene units bound to DNA may be responsible for a portion of the cytotoxic activity shown by the mitomycin family. Thus mitosene entities

can be intimately associated with all of the proposed segments of molecular activity of mitomycins.

Biosynthesis

Study of the biosynthesis of mitomycins using radio-labeled growth media has led to the assignment of biogenic precursors of the mitomycin family. This work has been reviewed by Remers¹ and extended by Hornemann¹⁵. The biosynthesis, summarized in Figure 5 for porfiromycin, involves construction mostly from various carbohydrate moieties. Carbon atoms 1, 2, 3, 9, 9a, 10 and their heteroatom substituents are derived from D-glucosamine. The glycolysis product, pyruvate, is the precursor to carbon atoms 5, 6 and 6a. The remainder of the quinone ring, carbon atoms 4a, 7, 8 and 8a, is derived from D-erythrose. N-Methyl and 9a-O-methyl groups have been shown to arise from the S-methyl group of methionine. Finally, the carbamate comes from the urea cycle intermediate, L-citrulline.

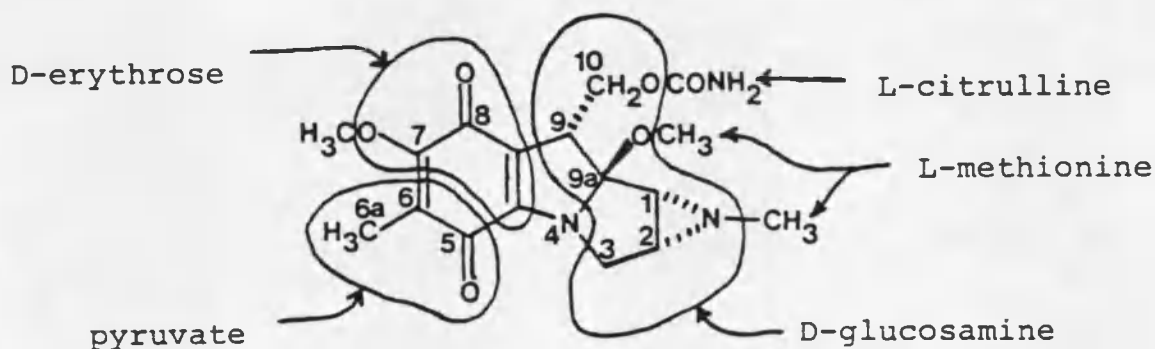
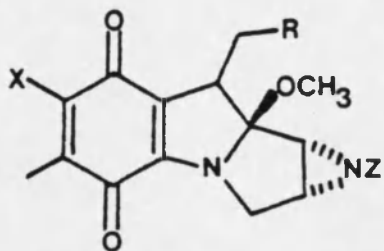


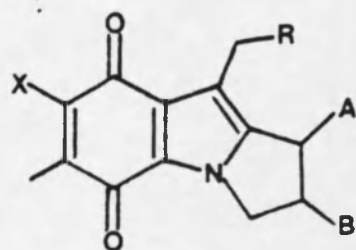
Figure 5: Biosynthetic precursors of porfiromycin.

Structure-Activity Relationships

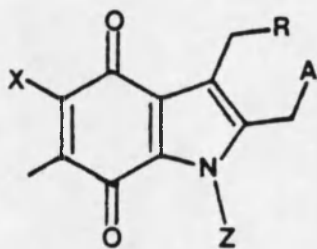
A large number of semisynthetic and totally synthetic analogs of mitomycins have been prepared. These are divided into several categories in Figure 6. The first group contains mitosane analogs which have been modified at any of the 1, 2, 7, or 10-positions. These compounds are principally available only through manipulation of the native compounds¹⁶⁻²⁴. A total synthesis of mitomycins by Kishi and co-workers²⁵⁻²⁷ has been achieved and although it is an elegant example of synthetic chemistry, it is an extremely long and difficult way to obtain analogs. Progress on a new route based on photochemical production of seco-mitosanes may hold promise for a shorter route to synthetic mitosanes²⁸⁻³⁰. The second category of analogs, the mitosenes closely resemble mitosanes except that they have been further aromatized to an indoloquinone system by the elimination of methanol from the 9 and 9a-positions. Mitosenes are readily available by a number of totally synthetic routes³¹⁻⁴⁰ or by conversion from native mitomycins^{16-18, 41-45}. The third group, the indoloquinones are simply mitosenes lacking the pyrrole ring. They are also readily available by total synthesis⁴⁶⁻⁵⁵. Two final groups of synthetic analogs which differ more markedly from the native mitomycins by their simplicity of structure are benzoquinones⁵⁶⁻⁶¹ and pyrroles⁶²⁻⁶⁵.



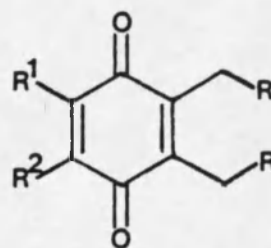
Mitosanes



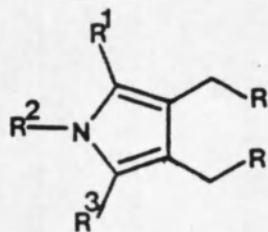
Mitosenes



Indoloquinones



Benzoquinones



Pyrroles

Figure 6: Analogs of mitomycins.


Due to the large number of mitomycin derivatives that have been prepared, detailed biological data from which structure-activity relationships are derived will not be presented. Some broad generalizations are offered for the purpose of designing analogs⁷. First, two alkylating functions are required for good antineoplastic activity even though monofunctional derivatives often show antibacterial action. Of the difunctional analogs prepared those mitosane and mitosene derivatives with the 1,2-aziridine system consistently show high levels of anti-tumor activity, a fact that may be related to observed lower pH in cancer cells compared to normal cells¹. Second, the reduction potential of the quinone is related to tumor cell specificity. This is based on the observation that the reducing power of cancer cells is often considerably different from normal cells. Ideally one could exploit such a difference to achieve selectivity against cancer cells. For examples, variation of the 7-substituent or elimination of the 9a-methoxy group result in altered quinone reduction potential. Third, increased water solubility increases activity against leukemias. Finally, aromatization as in mitosenes, indoloquinones, benzoquinones and pyrroles is compatible with activity. This is to be expected considering bioactivation theories. The challenge in developing analogs to mitomycin C is combining

the elements above in a way that maintains the high level of activity and potency of the parent compound.

CHEMISTRY

Target Compounds

The established high level of antineoplastic activity of a number of semisynthetic aziridinomitosenes⁶⁶ led to the choice of other substituted mitosenes as targets for novel analogs to the native mitomycins. In addition the synthetic mitosene, 1-acetoxy-7-methoxymitosene (17) had previously been shown to be active in both λ -bacteriophage induction and P-388 murine leukemia assays for anti-tumor activity³⁴. Although this latter compound was clearly inferior to the aziridinomitosenes and mitomycin C in terms of efficacy, it was felt that a thorough examination of alternative leaving groups in the 1-position held promise for better antineoplastic activity. It was also felt that alteration of the quinone reduction potential by variation of the 7-substituent could boost activity by enhancing the selectivity of such 1-substituted mitosenes against cancer cells. In mitosenes replacement of the 7-methoxy group with a 1-aziridinyl substituent showed a clear enhancement of activity^{66,67} so this substitution was considered as a secondary area for investigation. The above factors in mind, various 1-substituted-7-methoxymitosenes and 1-substituted-7-(1-aziridinyl)mitosenes were selected as target analogs. These are shown in Figure 7

where X = CH₃O or  and R = ester, carbamate or halogen. Such compounds have the ultimate advantage of being available by total synthesis.

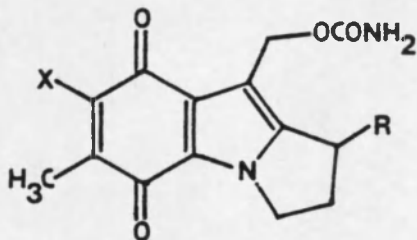


Figure 7: Target compounds.

Synthesis of Substituted Mitosenes

The synthetic route chosen (Figure 8) is a combination of the synthesis first proposed by Allen and co-workers³¹ in their synthesis of 7-methoxymitosene (18) and its later form as modified by Leadbetter and co-workers³³ for the synthesis of 1,2-disubstituted mitosenes. This work was extended to fit the desired modifications in the 1 and 7-positions. In addition major improvements were devised for the preparation of 14 and 15 from 12. Thus the reduction of 12 to 13 with tin and hydrochloric acid followed by treatment with potassium nitrosodisulfonate (Fremy's salt) gave 14 in 80% yield as compared to 39% yield reported in the literature³³. Selective reduction of the carboxaldehyde function of 14 with 9-borabicyclo-[3.3.1]-nonane (9-BBN) gave 15 at 70% yield in a process

that is more dependable than the documented method using sodium borohydride followed by ferric chloride regeneration of the quinone. These modifications make the synthesis much more attractive in lieu of its length and non-convergent nature. An overview of the synthesis shows elaboration of the indole nucleus by a Reissert approach with the addition of the pyrrolo ring by a sort of Dieckmann cyclization and decarbomethoxylation to give the tricyclic ketone, 8. Subsequently quinone, carbamate and 1-substituents are elaborated leading to the desired analogs, 17-25. Further modification then leads to 7-substituted analogs, 26,27.

Beginning with commercially available 2,5-dimethylphenol (1) the synthetic scheme progresses by nitrosation and subsequent oxidation to give the nitrophenol, 2. Next the phenolic hydroxyl group was converted to its methyl ether (3) via alkylation of its sodium salt with dimethyl sulfate. This anisole derivative has slightly acidic protons on the 5-methyl group which enables condensation with diethyl oxalate using potassium t-butoxide as a base. The product, a maroon colored potassium salt of the methylene group, was neutralized in boiling sodium bicarbonate solution, a process which also hydrolysed the remaining ester to give the phenylpyruvic acid derivative, 4 upon acidification. Reduction of the nitro group with

ferrous sulfate in ammonium hydroxide solution completes elaboration of the indole nucleus as the resulting amino group spontaneously condenses with the active pyruvate carbonyl, dehydrating to give the indolecarboxylic acid, 5. This last step has a particularly tedious and sloppy work-up due to production of large quantities of ferric oxides as by-products. Large scale work was greatly facilitated using the centrifugation procedure described in the Experimental chapter to separate ferric oxides instead of filtration procedures previously described³¹. Since yields in the neighborhood of 65% were possible by this method it was preferred over the simpler but lower yield zinc-acetic acid reduction reported by Leadbetter³³.

Before cyclization of the third ring, the indole carboxylic acid derivative was converted to its methyl ester (6) by a Fisher esterification in methanolic hydrogen chloride. The 94% yield reported for this reaction³¹ was difficult to reproduce but yields around 75% were readily obtained which was considered adequate for large scale work. Condensation with methyl acrylate using potassium t-butoxide to abstract the indolic proton afforded smooth cyclization to the β -keto ester, 7 which was subsequently decarbomethoxylated in refluxing 95% acetic acid to give the tricyclic ketone, 8. This intermediate is quite stable and a convenient dividing point between hundred gram scale synthesis and work on a more moderate

scale. The combined yield for these seven steps is 16% or approximately 77% per step.

Sodium borohydride reduction of the ketone, 8, to 9 proceeded very smoothly as did acetylation with acetic anhydride in pyridine to give 10 in excellent yield. The acid washing described in the literature³³ to remove excess pyridine is not recommended since 10 is unstable under acidic conditions. Instead filtration of the water precipitated product followed by water washing and vacuum drying was preferred. This procedure removed most if not all of the excess pyridine and any traces left did not adversely affect the next step. Neither the tricyclic alcohol, 9, or its acetate, 10, are stable for long periods of time at room temperature so refrigeration and prompt formylation are recommended. The Vilsmeier-Haack reaction was used to formylate the 9-position, giving 11. The traditional procedure, phosphorous oxychloride in dry N,N-dimethylformamide (DMF) followed by hydrolysis in cold saturated sodium acetate, gave good results. In addition a procedure using methylene chloride-pyridine as co-solvents was developed as a possible route to ¹³C labeled compounds in the 10-position. The second procedure gave better yields but was never employed with ¹³C-DMF. So long as highly purified 8 was used as starting material, none of these three steps required any purification and 8 could be converted to 11 with a yield of 75%.

Nitration of 11 with 90% nitric acid in acetic acid to give 12 was the first of three steps necessary for elaboration of the quinone. In this step it was found that purification by chromatography on silica gel with chloroform gave superior results both in terms of purity and yield compared to the ethanol recrystallization reported in the literature³³. This simple modification improved the yield from 70% to 85%. Newly developed procedures for the next two steps also improved the yield compared to literature methods. Reduction of the nitro group of 12 to the amine, 13, was accomplished with a tin-hydrochloric acid reduction in aqueous ethanol. The product, a pale yellow oil was stable enough to obtain spectral data not previously reported but it was not routinely isolated since the quinone is much more stable. Treatment of 13 with Fremy's salt in an acetone-0.3 M pH 6.0 phosphate buffer mixture gave the quinone, 14, as an orange solid after chromatography. Yields as high as 82% for these two steps combined were obtained from gram batches of 12.

The next step was initially the most difficult in the entire synthesis. It involved reduction of the aldehyde function of 14 with sodium borohydride. The quinone carbonyls are also reduced under these conditions and must be reoxidized to obtain 15. The difficulty lies in the extreme instability of the hydroquinone intermediate. In this form the 1-acetoxy substituent readily eliminates

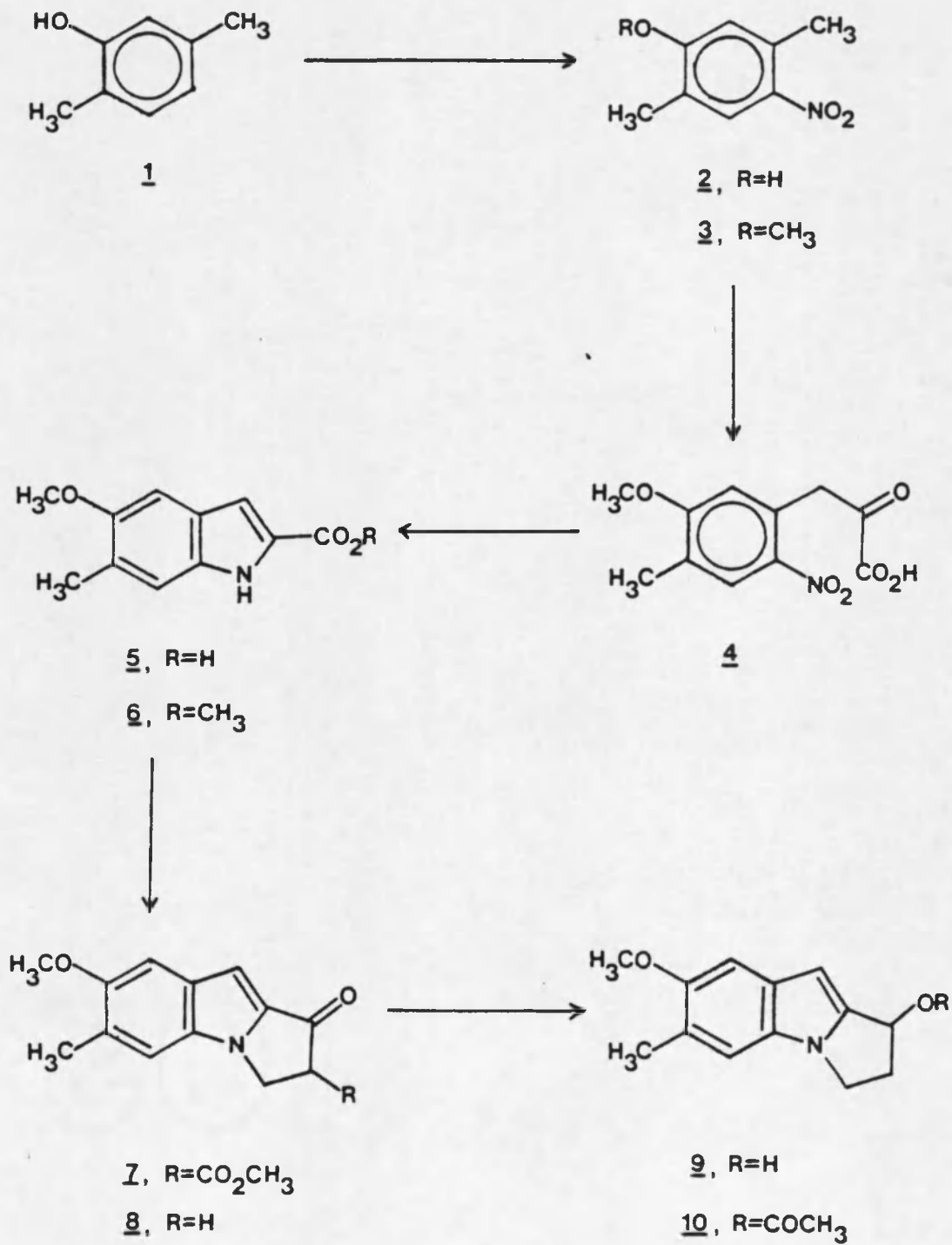


Figure 8: Mitosene synthesis

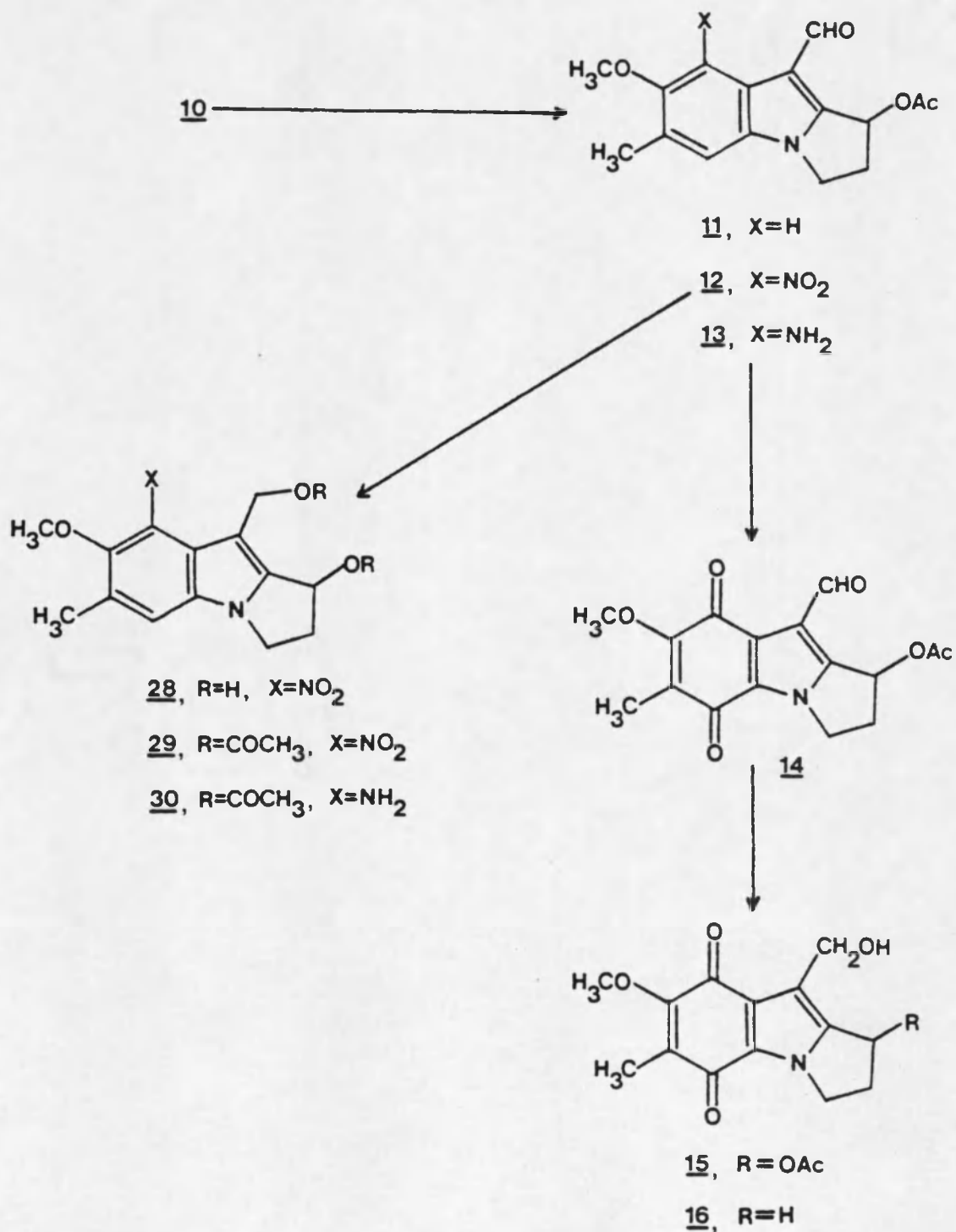


Figure 8--Continued: Mitosene synthesis

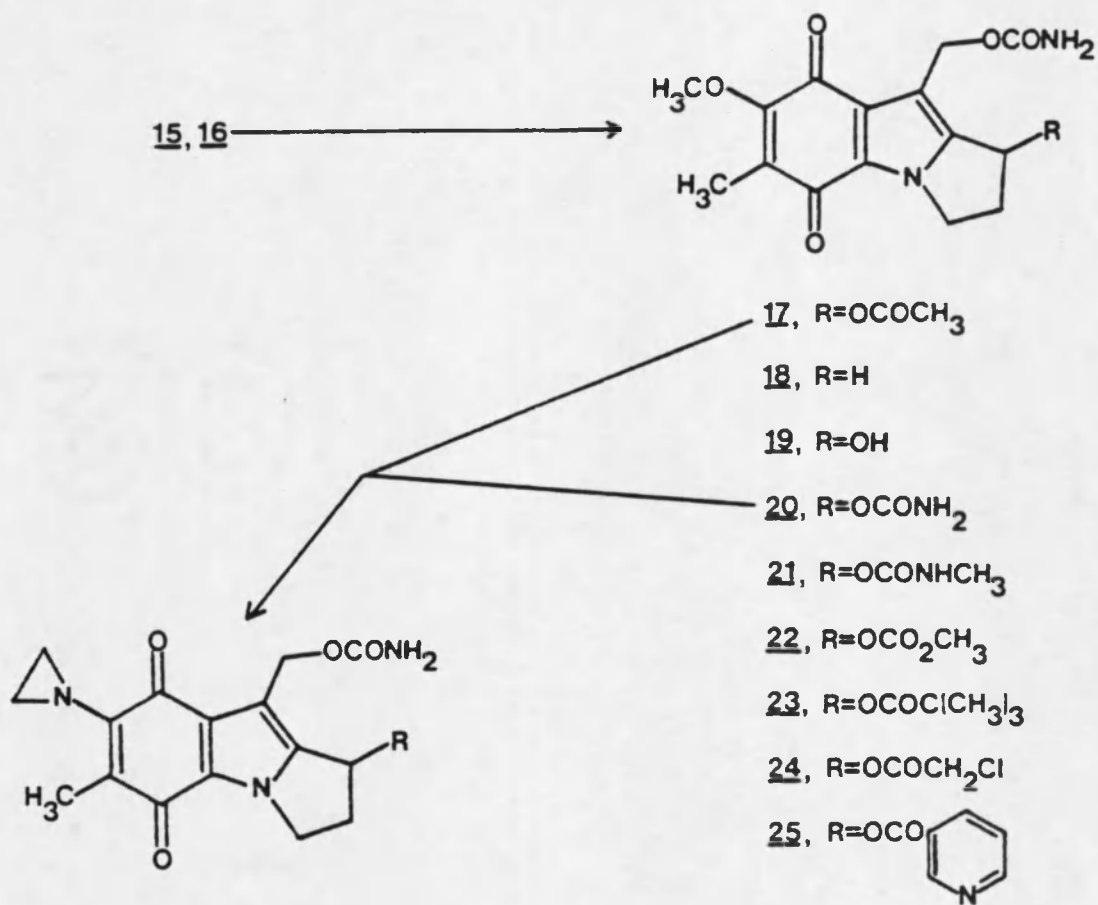


Figure 8--Continued: Mitosene Synthesis

leading to substantial decomposition if it is not quickly re-oxidized to the quinone. For a successful reaction it is essential to run the reduction in an oxygen free atmosphere until all of the orange quinone color has disappeared then re-oxidize the quinone, but not the 10-hydroxyl group, by addition of a ferric chloride solution. Ideally the reduction step should be as rapid as possible so that initially reduced molecules do not begin to decompose before all the starting material is consumed. For this reason a large excess of sodium borohydride was used. Presumably this is the reason why smaller scale reactions (200-300 mg) gave superior yields compared to large ones (over 500 mg). Even in the smaller batches a considerable variation in yield ranging from 35-65% was observed, most probably because of the difficulty in judging reaction completion from the color of the reaction. Another factor that was critical was the quality of sodium borohydride used. A fresh bottle that was dry and granular gave superior results to old, lumpy borohydride. Buffering with small amounts of acetic acid moderated inconsistencies based on sodium borohydride but did not eliminate occasional low yields.

Beside the desired product, 15, another product, 16, was present in small amounts. This deacetoxy derivative probably results from elimination of acetate at the hydroquinone stage and capture of hydride by the resulting

carbonium species. Compound 16 tended to be more prevalent when the overall yield was low and ranged from 5-15% of the isolated product. Separation of 15 and 16 was found to be difficult by gravity chromatography but was easily accomplished using preparative scale HPLC. It was generally easier, however, to delay separation until after the carbamates had been elaborated and 1-acetoxy substituent hydrolysed. The resulting mixture of 18 and 19 was easily separated by gravity chromatography.

Because of the difficulty of reducing the 10-aldehyde in the presence of a quinone an attempt was made to circumvent the problem by reduction of the aldehyde function at an earlier stage in the synthesis. Thus treatment of 12 with sodium borohydride, protection of the resulting hydroxyl group and subsequent conversion to the quinone was attempted. The logic behind such a sequence was that the amine, 30, might be more stable than the hydroquinone of 15. This, however, did not prove to be the case. Reduction of 12 with sodium borohydride gave, somewhat surprisingly, the dihydroxy adduct, 28. The mechanism for hydrolysis of the 1-acetate was not investigated but acetylation clearly resulted in functionalization of both hydroxyl groups to give 29. The conversion of 12 to 29 is not a very good process in terms of yield or reproducibility. In addition the reduction of the nitro group with zinc in dilute hydrochloric acid proved to be

troublesome since 30 was very unstable, decomposing at room temperature in deuteriochloroform while attempting to obtain an NMR spectrum. The combined difficulties of this route made it less attractive than that already established so it was abandoned without attempting the conversion of 30 to the corresponding quinone with Fremy's salt.

In lieu of the difficulties described in the preceding three paragraphs an attempt was made to selectively reduce only the aldehyde function of 14. Analysis of the problem indicated that perhaps a sterically hindered reducing agent would prefer attack at the aldehyde over the more hindered quinone carbonyls. 9-BBN was investigated for this purpose and was found to give a surprising degree of chemoselectivity when limited quantities were used. Thus reduction of 14 with two equivalents of 9-BBN in tetrahydrofuran consistently gave 15 at approximately 70% yield. With large excesses of 9-BBN and longer reaction time the quinone was also reduced. The reaction is therefore not totally selective but it can be controlled to achieve the desired result in good yield. The consistent results obtained by this method make it the preferred route for conversion of 14 to 15.

Once the 10-hydroxy adduct, 15, was obtained, conversion to its carbamate, 17, was easily accomplished using a two step procedure of acylation with phenyl chloroformate followed by ammonolysis of the phenolic ester

linkage in the intermediate phenyl carbonate product. It was necessary to carry out the latter step in methylene chloride since ammonia in more polar solvents displaced the 7-methoxy substituent. Addition of the carbamate moiety could be accomplished with yields in excess of 90%, completing the synthesis of 1-acetoxy-7-methoxymitosene (17). The corresponding 1-hydroxy derivative, 19, was easily obtained by hydrolysis of the acetate with ammonium hydroxide in methanol-water solution. This was the key intermediate from which the 1-substituted mitosenes (20-25) were synthesized.

Functionalization of the 1-position was accomplished by acylation of its hydroxyl group either with an acid chloride or isocyanate. Unfortunately this was not as easy to achieve as with the 1-hydroxyl group earlier in the synthesis. The difficulty of acylation at the 1-position is caused by two basic problems. The first is that the 1-hydroxyl group is not very reactive toward acylating agents. A number of factors contribute to this low reactivity including the low nucleophilicity of a secondary, benzylic-like hydroxyl group, steric crowding from the 10-carbamoyl substituent and intramolecular hydrogen bonding between carbamoyl and hydroxyl groups. The second problem is that when the 1-acyl group formed was a good leaving group the product was often unstable. In fact, products with desirable 1-substituents such as

trifluoroacetoxy and methanesulfonyl could not be isolated. Also a problem in this regard was the fact that good leaving groups were often displaced by pyridine used as a catalyst in the acylation. Thus with methanesulfonyl chloride, chloroacetyl chloride and trimethylacetyl chloride the initially formed esters were converted to water soluble alkyipyridinium salts. This property has previously been demonstrated with indoloquinone analogs⁴⁹. For this reason a non-nucleophilic base such as potassium carbonate had to be used, a fact that undoubtedly lowers the yield since heterogeneous bases are less efficient in terms of catalysis. In general the yield for introduction of a 1-substituent was not high but usually it was adequate to generate analogs for biological testing (40-65%). This made the total yield for the synthesis of a 1-substituted mitosene from 2,5-dimethyl phenol about 1.5% which was considered to be quite good considering the fact that twenty-two steps were involved.

Two of the 1-substituted derivatives, 17 and 20, were subsequently converted to their 7-(1-aziridinyl) counterparts, 26 and 27, respectively. This was easily accomplished by treatment with ethylenimine in anhydrous methanol or DMF to give 7-substitution in a matter of a few hours at room temperature with high yields. This reaction can be described as an aminolysis of a vinylogous ester. It is interesting to note that the vinylogous ester

is more reactive to amines than either acetate or carbamate, while the acetate is the most reactive toward attack by hydroxide. Also of interest was the observation that conversion of 7-methoxy derivatives to 7-(1-aziridinyl) derivatives resulted in a marked color change from yellow-orange to burgundy, demonstrating the effect of the 7-substituent on the electron density of the quinone chromophore.

Aside from the original target compounds two other mitosenes were prepared. The chemistry for these two compounds is shown in Figure 9. The oxime, 32, was prepared for antineoplastic testing based on a previously discovered high level of antibacterial activity⁶⁸. 7-Methoxy-1-hydroxymitosene (19) was oxidized with manganese dioxide according to literature procedures³³. This oxidation could not be effected with tetrabutylammonium dichromate⁶⁹, presumably for the same reasons that inhibit 1-acylation. The ketone, 31, was converted to its oxime, 32, with hydroxylamine hydrochloride in methylene chloride-methanol-pyridine solution.

The second miscellaneous compound that was prepared for biological testing was the 10-chloro adduct, 36. It was obtained by a last ditch effort to prepare 37. Treatment of 19 with thionyl chloride or phosphorous oxychloride failed to give a 1-chloro substituent despite literature precedent with indoloquinone analogs⁴⁹. Hence an attempt to perform this conversion by treating 17 with dry

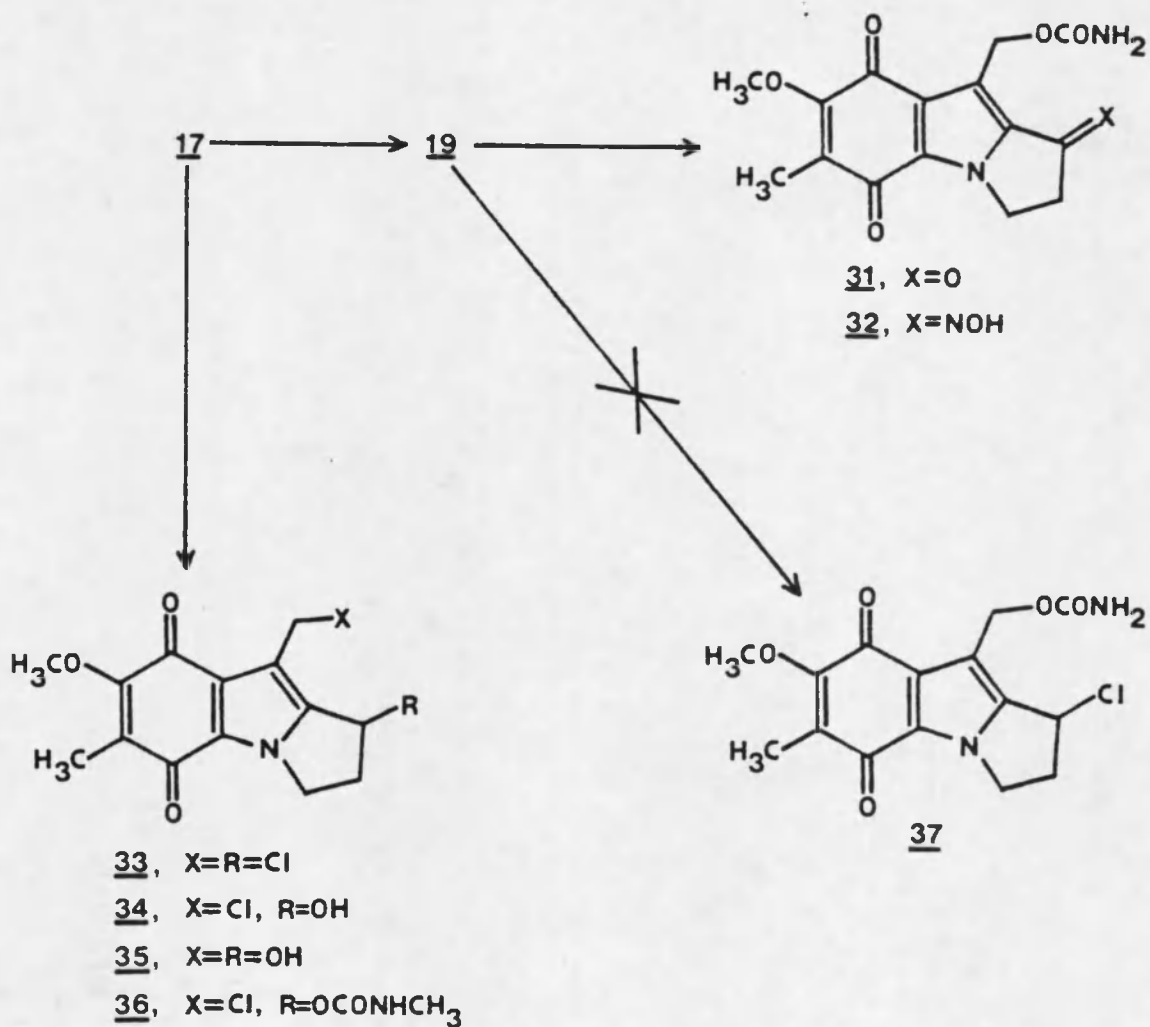


Figure 9: Miscellaneous mitosenes

hydrogen chloride was made. After work-up and chromatography 34 was isolated in good yield. It is theorized that the dichloro compound, 33, was the original product and was hydrolysed by water on the silica gel during chromatography. When very dry adsorbent was used the product eluted much more rapidly and decomposed before identification could be

made. Slightly wetter silica gel hydrolysed both chloro functions giving 35. Treatment of 34 with methyl isocyanate gave a good yield of 36 so long as oven-dried silica gel was used in purification. This reaction proceeded more rapidly than the analogous conversion of 19 to 21, further demonstrating the dampening effect of the 10-carbamate on reactivity of the 1-hydroxyl group.

Mass Spectral Fragmentation

During the course of structure verification of the various substituted mitosenes a great deal of mass spectral data was generated. It is reported here for three reasons: First, although mass spectra for the native mitosenes and their corresponding aziridinomitosenes have been reported⁷⁰, the fragmentation patterns for mitosenes without a 1,2-aziridine have not been previously described. Second, in addition to confirming structure, the mass spectral data observed confirm the reactivity of the groups in the 1- and 10-positions. Third, the characteristic fragmentation of mitosenes could be quite a valuable tool for determining in vivo binding sites for mitomycins since mitosenes have been implicated as the unit of alkylation and the sensitivity of mass spectral analysis would surely be required for in vivo study.

Low resolution mass spectra and a partial listing of important peaks are provided for the mitosene analogs in

Appendix A. In all but one of the compounds analysed a molecular ion was visible with electron impact ionization of vapor produced by heating a solid sample probe. The molecular ion is characteristically a small peak with a relative intensity less than ten percent. The usual pattern of breakdown consists of a few predictable low intensity ions until one reaches the base peak which is dependant on the 7-substituent but independant of the 1- and 10-substituents. Below the base peak there are usually one or two identifiable ions of moderate intensity and many low intensity ions that are difficult to identify. This data is presented schematically in Figures 10, 11 and 12. As a specific example, the structures resulting from fragmentation of 1-Acetoxy-7-methoxymitosene are shown in Figure 10. Fragment structures for the various other mitosenes follow by close analogy. General representations of the mass spectral fragmentation patterns for 7-methoxy derivatives and 7-(1-aziridinyl) derivatives are summarized in Figures 11 and 12, respectively.

Concentrating on 7-methoxymitosene derivatives (Figure 11) one can see that decomposition of the molecular ion can take place by loss of either the 10- or 1-substituent. This is an interesting parallel to the biological activity proposed to be associated with the functional groups at these positions. The 10-carbamate is lost either

by elimination of isocyanic acid, carbamic acid or carbamate radical. Similarly the 1-substituent, for example an acetate, can be eliminated via the ketene, carboxylic acid or carboxylate radical. Combinations of loss of ketene plus carbamic acid or isocyanic acid plus carboxylic acid lead to the most abundant ion at m/z 259. In those compounds that can not lose a ketene or an isocyanate from the 1-position, for example 22, 24 and 25, the pattern is simplified somewhat as shown by the dashed arrows. The base peak remains at m/z 259, however, since it can arise from two sources. Another characteristic peak for the 7-methoxy compounds is m/z 244 which is nearly always the second most intense ion. It occurs due to elimination of a methyl radical from the base peak. Occasionally one can see the combination of loss of isocyanic acid from the 10-position plus loss of ketene (or isocyanate) from the 1-position but this fragment gives a low intensity peak and can not be seen consistently.

In the case of 7-(1-aziridinyl)mitosenes (Figure 12) the fragmentation pattern is the same except that due to the weight difference of the 7-substituent, the base peak now becomes m/z 270. In addition one sees elimination of ethyl radical or ethylene from the base peak leading to the second most intense ion. One would thus expect that other 7-substituents would also not affect decomposition of the

ion but merely shift the m/z value of the base peak accordingly. Hence 7-aminomitosene derivatives, for example, should give a base peak at m/z 244.

Replacement of the 10-carbamoyl function with a chlorine atom alters the mass spectral pattern only minimally as shown by the data for 36 (Appendix A). One simply sees the loss of chlorine radical and hydrochloric acid instead of the usual carbamate radical and carbamic acid. Loss of hydrochloric acid plus methyl isocyanate gives a base peak of m/z 259 in direct analogy to the previously described 7-methoxymitosene derivatives. Because of the similarity of cleavages at the 1- and 10-positions one would expect that if it was available, 7-methoxy-1-chloromitosene would behave accordingly giving a similar fragmentation to 36. This is an interesting proposition since it could also be extended to cover alkylated biomolecules. Monofunctional alkylation of a nucleotide, for instance, would give predictable fragment ions based on the mitosene portion of the alkylated product so long as it is a heteroatom that is alkylated. Bifunctional alkylation would not necessarily follow this rule unless one of the alkylations was on oxygen. Alkylation of two nitrogen atoms, for example, would obviously be expected to alter the composition of the mitosene derived base peak. Thus by focusing on mitosene fragments a great deal could be learned about alkylation sites on DNA.

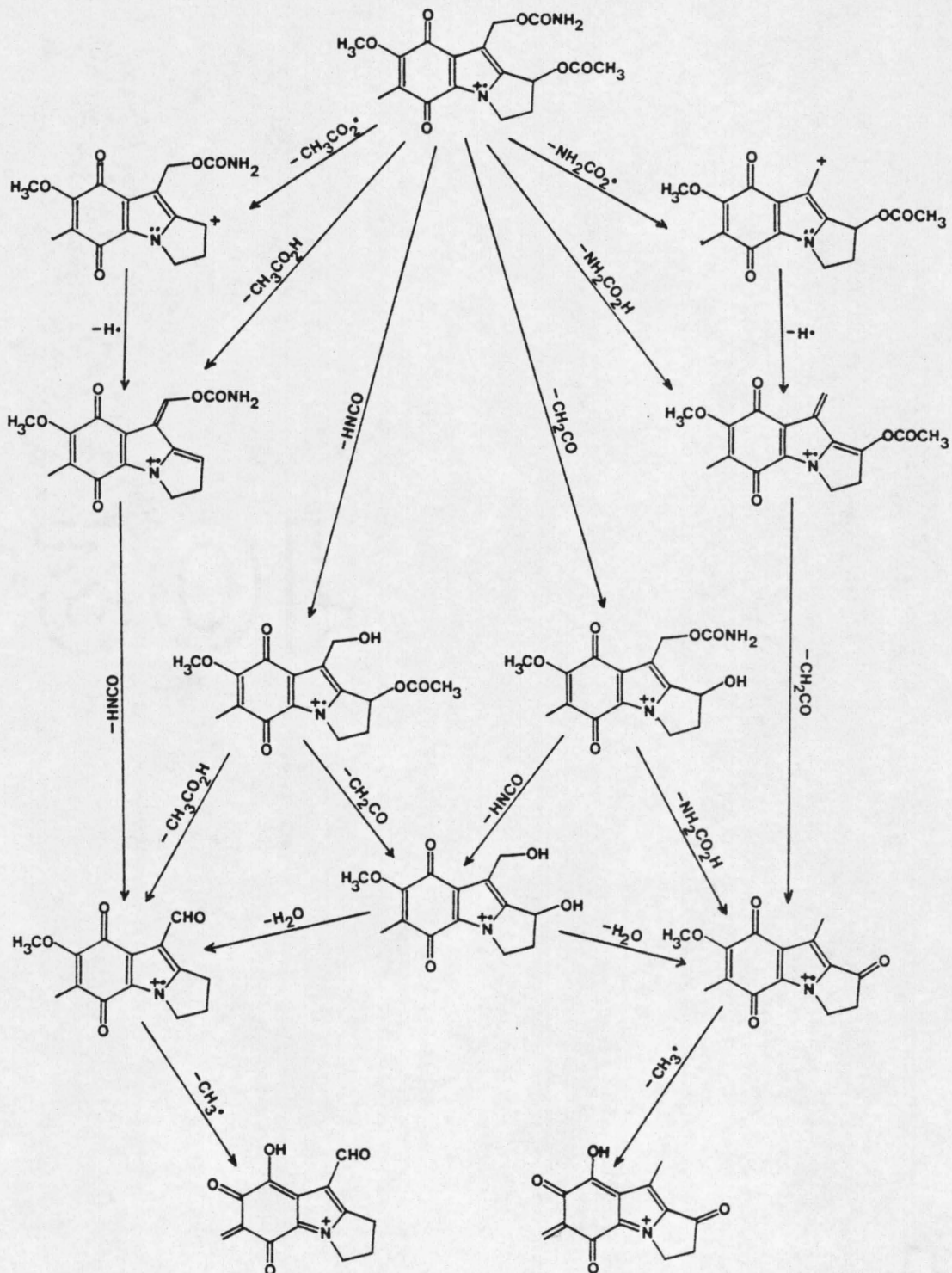


Figure 10: Fragment structures for 1-Acetoxy-7-methoxymitosene

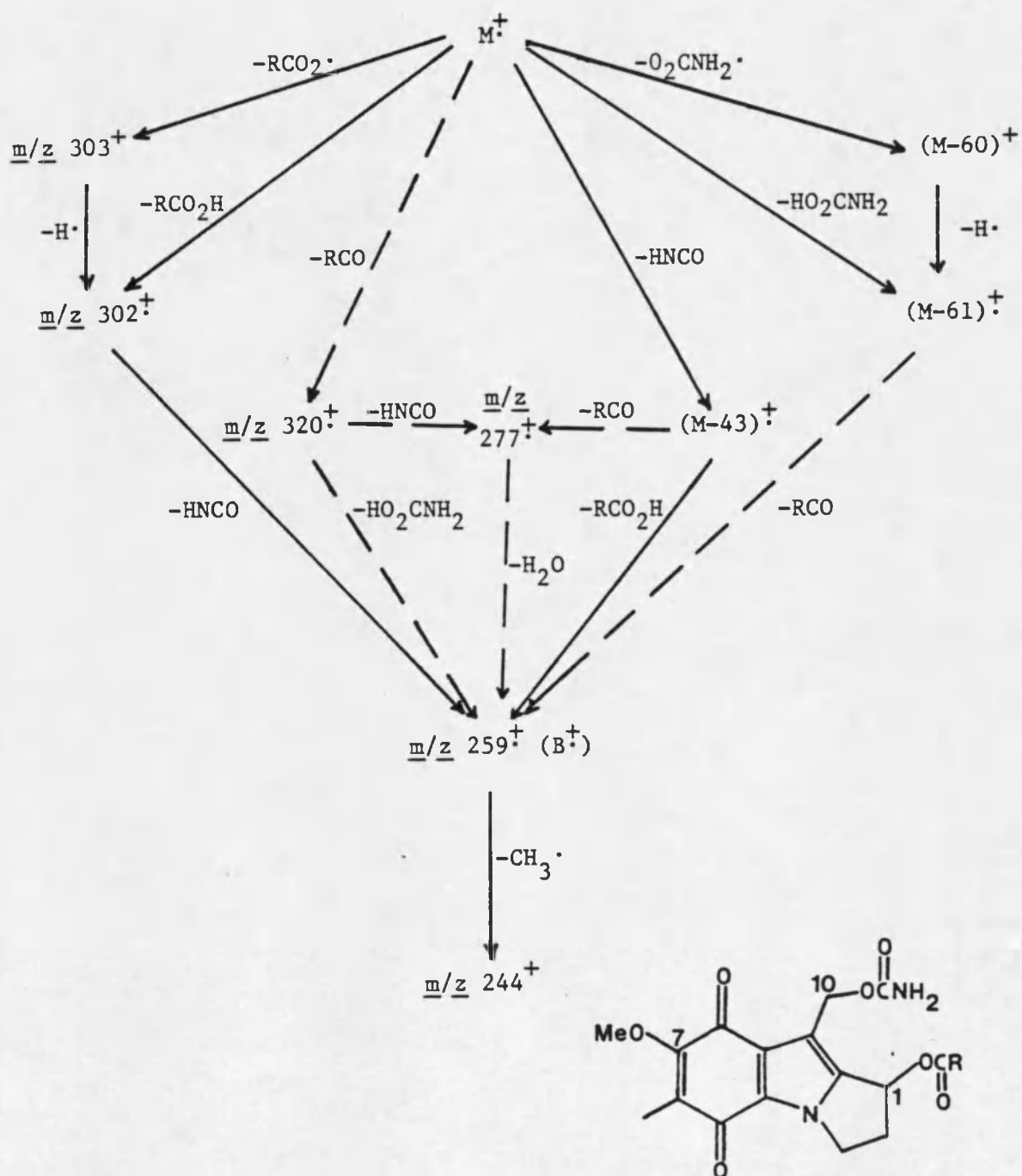


Figure 11: 7-Methoxy derivative fragmentation

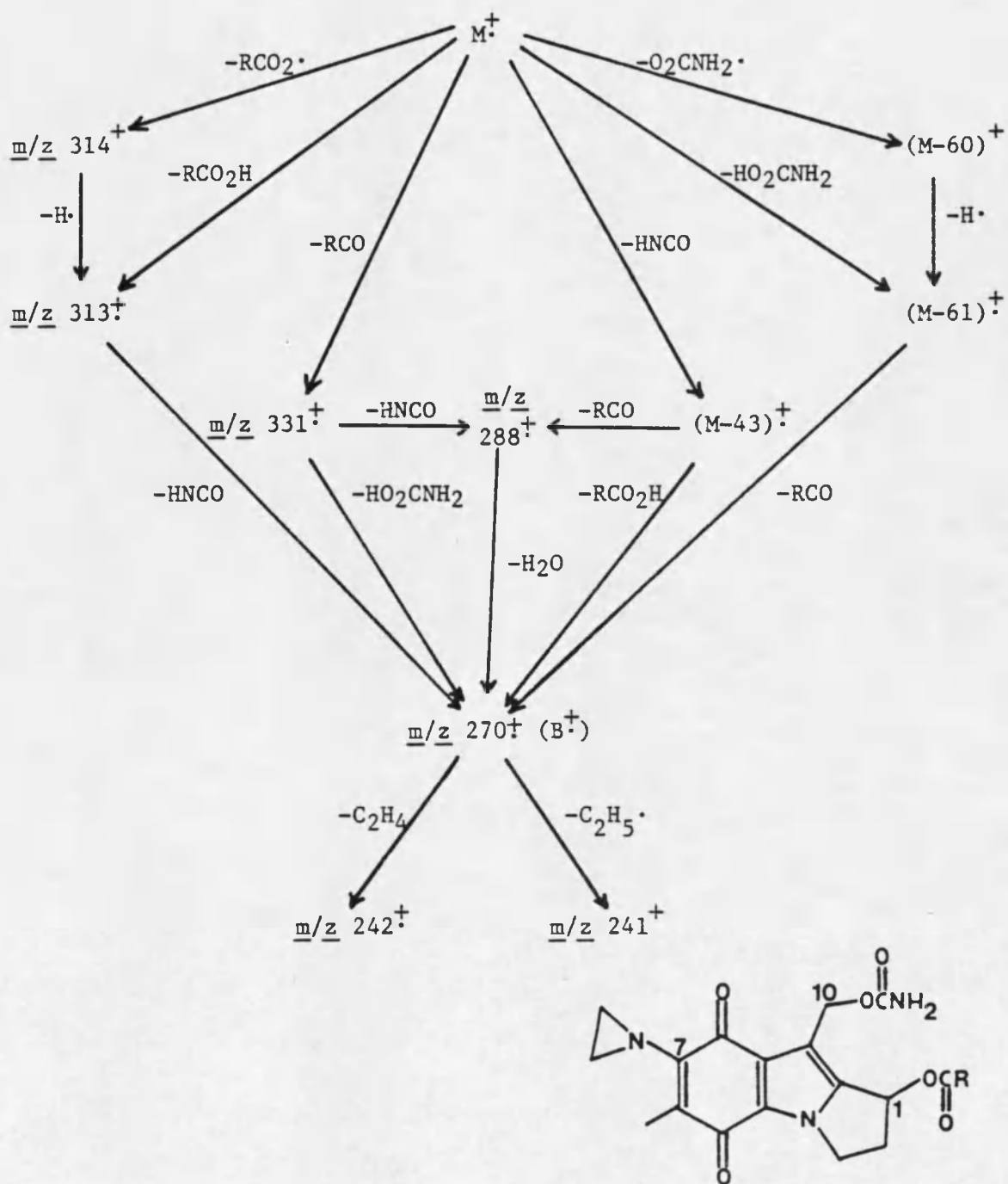


Figure 12: 7-(1-Aziridinyl) derivative fragmentation

Mitosane vs. Mitosene

As an addendum to the synthetic work completed the following is a summary of the chemical properties of mitosenes in relation to their parent mitosanes.

Acid Reactions

The extent to which the chemical behavior of mitosenes resembles that of mitosanes depends largely upon the presence or lack of an aziridine ring. Mitosanes and mitosenes with 1,2-aziridino functions are easily hydrolysed by dilute acid solutions resulting in 1,2-disubstituted mitosenes known as apomitomycins³⁷ (Figure 13.1, 13.2). In addition, if other nucleophiles are present with an acid catalyst they can substitute at the 1-position. One very interesting example of this type of reaction is the capture of phosphates in slightly acidic media⁸. Without the aziridine ring in the 1,2-positions mitosenes are generally much less reactive in dilute acid as illustrated by 7-methoxy-1-acetoxymitosene (Figure 13.3). The exception to this rule is the compound 25 (Figure 13.4) which is hydrolysed to 19 rapidly in 0.05N hydrochloric acid (see Experimental). The lability of the nicotinyl group in acid is probably a reflection of the inductive effect of protonation at the pyridine nitrogen making the zwitterionic form of nicotinic acid a good leaving group.

It is interesting to note that there is a 4:1 ratio of cis to trans apomitomycin when N-methyl-mitomycin A (Figure 13.1) is hydrolysed in 0.05 N hydrochloric acid³⁸. This stereochemical preference is also seen with other mitosanes and is contrary to what might be expected compared to acid hydrolysis of aziridines on other ring systems where the trans product nearly always predominates⁷¹. Surprisingly N-methyl-aziridinomitosenone A (Figure 13.2) also gave a preference for the cis apomitomycin, however the ratio was reduced to 3:1. This result seems to indicate that the orientation of the 9a-methoxyl group has only a small influence on the stereochemistry of aziridine opening in acid. The conformation of the 9-carbamoyl-methyl substituent would thus seem to be the only conceivable factor controlling this stereochemical preference. One might expect a β -directed conformation for the carbamate due to the α -orientation of the aziridine ring. Such a conformation could inhibit attack of a nucleophile from the β -face at the 1-position and hence the preference for cis product.

In much stronger acid other functional groups are also subject to hydrolysis. With mitosanes (Figure 13.5) the 7-amino and 10-carbamoyl functions can both be hydrolysed with the 7-amino group being the more labile of the two^{1,18,40}. Strongly acidic conditions also affect mitosenes inert to dilute acid. 7-Methoxy-1-acetoxymitosene (Figure 13.6) shows acid lability of both the 1-acetoxy and

10-carbamoyl groups giving an unstable dichloro adduct by treatment with anhydrous hydrogen chloride which is readily hydrolysed by small amounts of water on silica gel. In summary, all mitosanes and mitosenes are labile in strong acid but under milder conditions those compounds lacking a 1,2-aziridine are reasonably unreactive.

Base Reactions

Opposed to the result of acid hydrolysis, mitosanes and mitosenes with 1,2-aziridines are quite stable with respect to this functionality. In addition the 9a-methoxy group of mitosanes is not eliminated under basic conditions. The focus of hydrolysis shifts to the 7-position, specifically when there is a 7-amino group present in the molecule. As shown in Figure 13.7, the 7-amino group of mitomycin C can be hydrolysed in preference to the 10-carbamoyl substituent with sodium hydroxide in methanol¹⁸. One can view this as the hydrolysis of a vinylogous amide to a vinylogous carboxylic acid which exists as a salt in solution until neutralized. More forcing conditions such as prolonged hydrolysis with sodium hydroxide²³ or treatment with sodium methoxide^{16,24} results in the hydrolysis of the carbamate function to give 10-decarbamoyl-mitosanes. One would expect aziridinomitosenes C to parallel this behavior but basic hydrolysis of this compound has not been reported. The hydrolysis of the 7-amino group of mitomycin C is a useful

synthetic conversion since treatment of the 7-hydroxy adduct with diazomethane gives mitomycin A which is difficult to obtain by fermentation^{18,19}.

With a substrate such as 7-methoxy-1-acetoxymitosene (Figure 13.8) one sees preferential hydrolysis of the acetate ester under relatively mild conditions such as ammonium hydroxide in methanol³³. Presumably both the 7-methoxy and 10-carbamoyl groups are also base labile under more rigorous conditions but this assumption has not been substantiated since there is little need for such a synthetic conversion.

Weaker but more nucleophilic bases like amines show slightly different activity against mitosanes (Figure 13.9). Only the 7-position is labile to amines and in order to be displaced it must be 7-methoxy and the reaction carried out in a polar solvent like methanol or DMF. For example, mitomycin A treated with ethylenimine in methanol gives its 7-(1-aziridiny) counterpart. As previously discussed this is a useful reaction since it is one way to vary the reduction potential of the quinone.

Somewhat surprisingly the same displacement occurs with 7-methoxy-1-acetoxymitosene (Figure 13.10) in preference to attack at the 1-acetoxy substituent. Thus ethylene imine in methanol attacks the vinyllogous ester of the 7-position first whereas ammonium hydroxide in methanol attacks the acetate ester first. The change in locus of activity with nucleophile type is quite interesting and useful

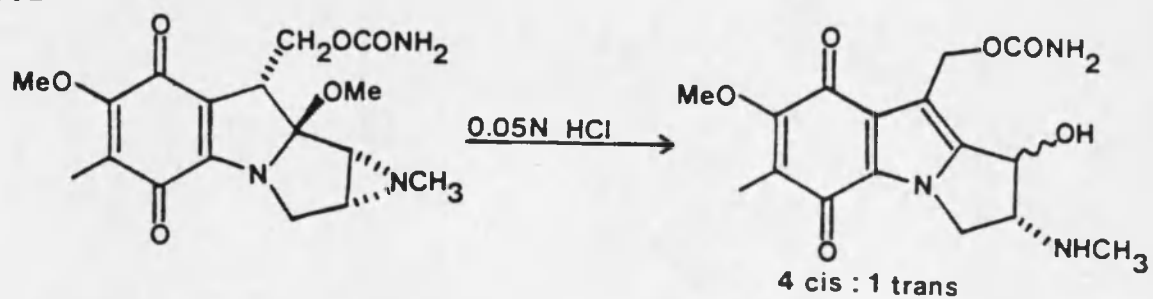
synthetically. Prolonged treatment with amines will eventually lyse the acetate to give the 1-hydroxy compound but this reaction is clearly slower than that at the 7-position (see Experimental). It is thus possible to achieve considerable chemoselectivity in reactions of mitosanes and mitosenes with bases by selection of suitable substrate, solvent and base.

Reduction

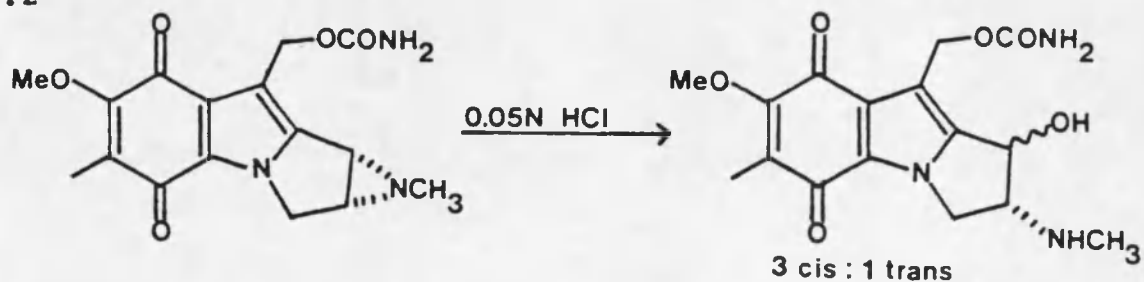
Given the fact that biological reduction is a proposed source of alkylating activity for mitosanes and mitosenes considerable modification would be expected upon chemical reduction. In both species reduction of the quinone ultimately leads to complete decomposition to many unidentified products unless it is accompanied by prompt re-oxidation of the hydroquinone intermediates. Even under the latter conditions it is difficult to get a good yield of any of the products. Depending upon the method used reduction followed by prompt re-oxidation can give a variety of chemical modifications. By reduction of the quinone the 4-nitrogen atom's electron pair is freed from conjugation with the 8-carbonyl and is able to participate in the elimination of a mitosane 9a-methoxy group. Thus, reduction of mitosanes with sodium borohydride followed by oxidation results in demethoxymitosanes (Figure 13.11) as hydride is captured by an intermediate iminium ion²⁴. Catalytic

hydrogenation followed by air oxidation converts mitosanes to aziridinomitosenes by elimination of methanol (Figure 13.12). This procedure has been developed to be the only useful route to aziridinomitosenes with yields ranging from 32% for the conversion of N-methyl-mitomycin A to N-methyl-aziridinomitosene A⁴¹ to about 50% for the conversion of mitomycin A to aziridinomitosene A⁶⁶. Finally, reduction of mitosanes with sodium hydrosulfite in the presence of potassium ethylxanthate followed by re-oxidation with oxygen gives two mitosene products (Figure 13.13) in which ethylxanthate moieties have been trapped⁹. One is the result of bifunctional alkylation and the other monofunctional alkylation at the 1-position. Analogous to this sort of behavior is that of mitosenes such as 14 when reduced by sodium borohydride (Figure 13.14). Partial elimination of the 1-acetoxy substituent accompanies reduction of the quinone and aldehyde so that upon re-oxidation of the quinone with ferric chloride one can isolate small quantities of 16 in addition to 15 as a result of capture of hydride at the 1-position.

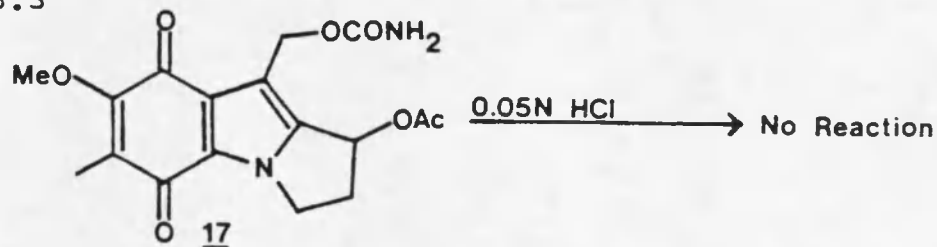
13.1



13.2



13.3



13.4

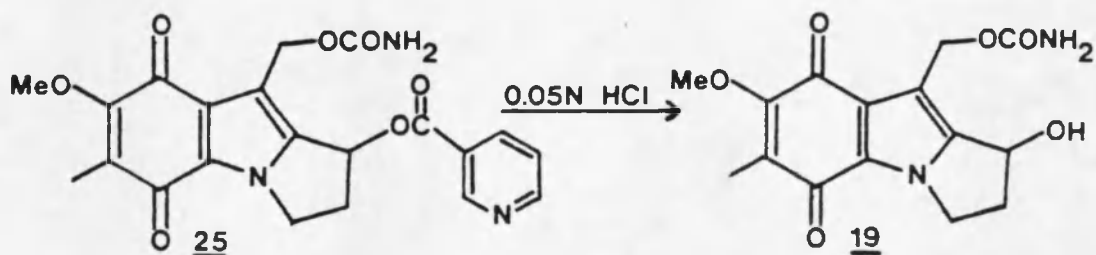
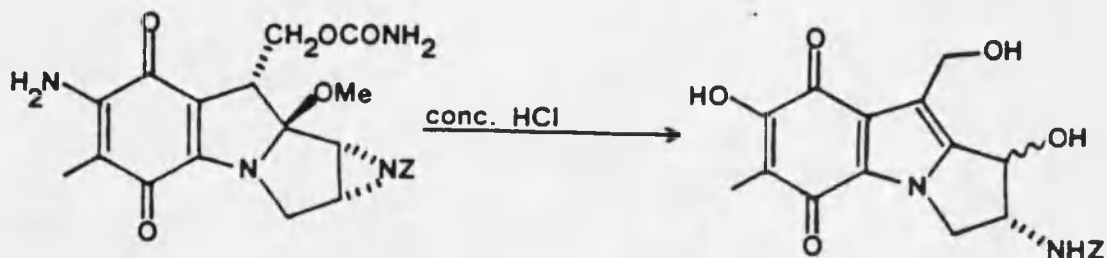
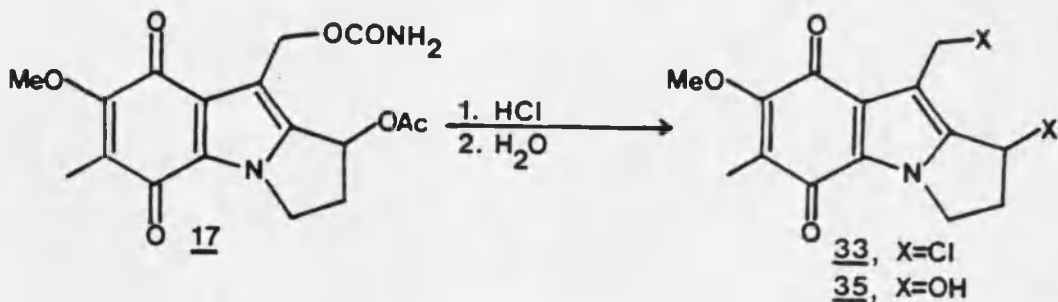


Figure 13: Reactions of mitosanes and mitosenes

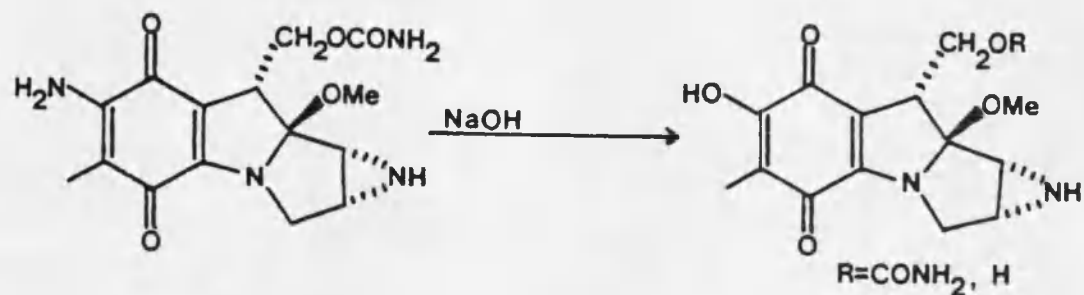
13.5



13.6



13.7



13.8

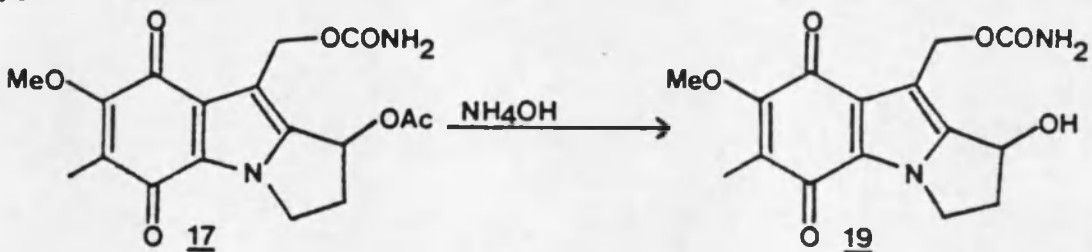
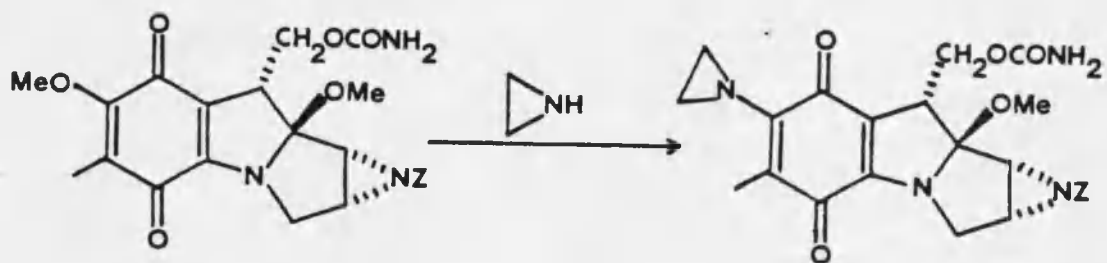
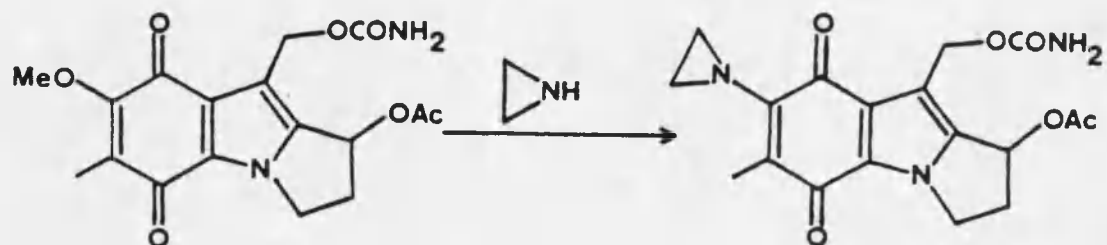


Figure 13--Continued:
Reactions of mitosanes and mitosenes

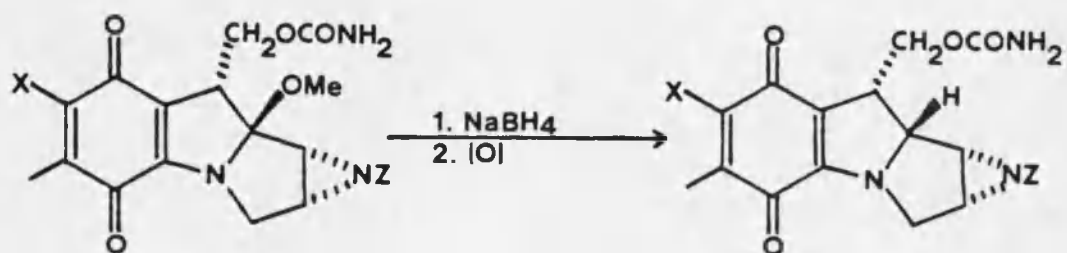
13.9



13.10



13.11



13.12

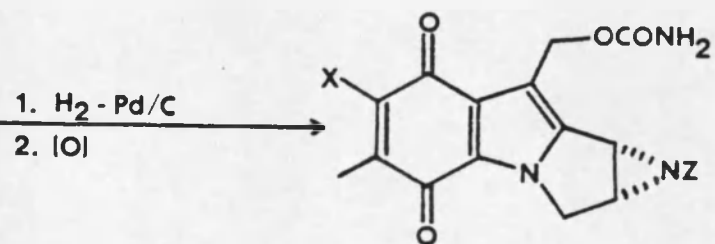
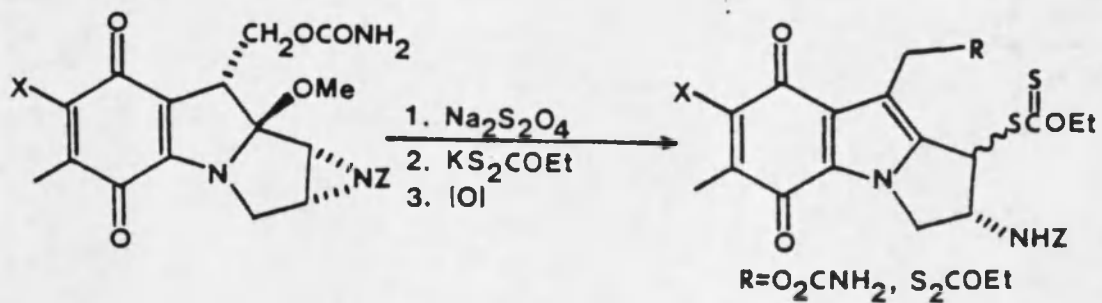


Figure 13--Continued:
Reactions of mitosanes and mitosenes

13.13



13.14

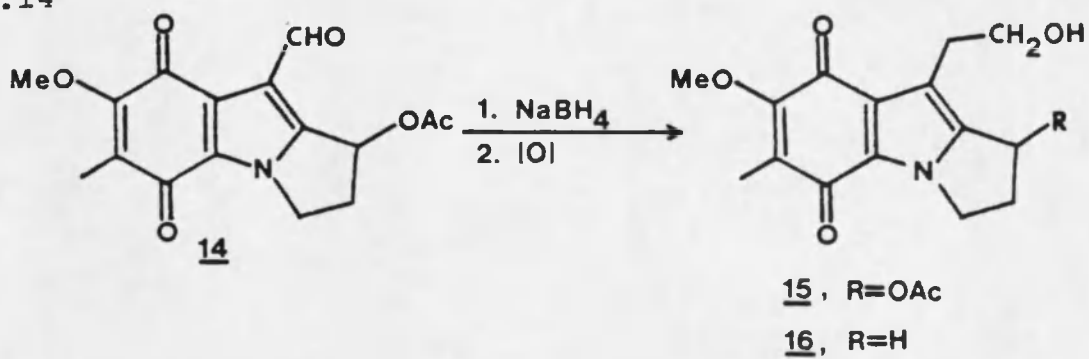


Figure 13--Continued:
 Reactions of mitosanes and mitosenes

ANTINEOPLASTIC ACTIVITY AND DISCUSSION

Activity Screening

The initial screen employed to detect antineoplastic activity of the new mitosene analogs was the murine lymphocytic leukemia P388 assay. Details of the standard protocol approved by the National Cancer Institute (NCI) have been published⁷⁵ so only a brief summary of the procedure will be presented. Basically it involves comparison of survival time of test mice, inoculated with a specific type of cancer cells and treated with an antitumor agent, to control mice that have been inoculated with the same line of cancer cells but receive no treatment. In addition to the untreated control there is a positive control that is treated with a standard drug, 5-flourouracil (NSC-19893) to serve as an indicator of tumor virulence.

The animals used in the screen are either BDF₁ or CDF₁ mice. Within a particular experiment mice of all one sex are used for both test and control groups. They must be within a 3 g weight range with a minimum weight of 18 g for males and 17 g for females. Groups of six mice are used for each dosage level tested and for the positive control. The number of mice in the untreated control varies according to the number of test groups.

The measure of activity for this assay is the median survival time (MST) of treated vs. control animals expressed as $\%T/C = MST(\text{test})/MST(\text{control}) \times 100$. A T/C value greater or equal to 125 on the initial screen indicates antineoplastic activity worthy of further consideration. Any test resulting in a T/C less than or equal to 85 denotes a toxic reaction. Acceptable MST for control animals is 9-14 days. Usually treatment starts 24 hours after implantation of tumor cells and continues 9 days at equal doses. An alternative treatment regimen consists of a single dose 24 hours after tumor cell transplant. Confirmed antineoplastic activity is defined as a T/C value greater or equal to 125 reproduced in two separate laboratories.

The testing schedule is summarized below on a daily basis.

Day 0: Tumor transfer is achieved by intraperitoneal (ip) injection of ascitic fluid containing 1×10^6 cells diluted to 0.1 ml.

Day 1: Animals are randomized into test and control groups. The mean animal weight is computed for each group and treatment is started for test groups by ip injection of the drug. Control mice receive a saline injection.

Day 2-9: Daily ip injection of drug is performed and deaths recorded. On day 5 the mean weight is computed for each group and those test groups with less than 65% survivors are eliminated from further consideration.

Day 20: If there are no survivors in the control group the %T/C is calculated for each test group.

Day 30: All survivors are killed and %T/C evaluated.

Screen Results

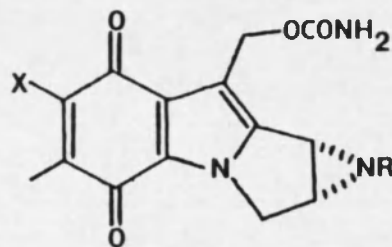
Activities of the substituted mitosenes against P388 leukemia are summarized in Table 1. Testing was performed under the supervision of Dr. William T. Bradner of Bristol Laboratories and follows the NCI protocol with the single dose regimen. In addition, mitomycin C was tested in each experiment as a second measure of tumor virulence and for the basis of comparison to the analogs. The maximum T/C value for mitomycin C is reported in parentheses next to each analog. Note that the value varies from 181 to 244 depending upon the particular experiment. CDF₁ female mice were used in all experiments. Optimum dose (O.D.) is the maximum dose at which the highest T/C value is seen. Minimal effective dose (M.E.D.) is the lowest dose that gives a T/C greater or equal to 125. Therapeutic ratio (T.R.) is O.D. divided by M.E.D.

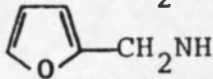
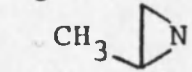
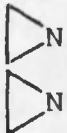
A group of semisynthetic aziridinomitosenes has also been tested in the P388 assay. These compounds were prepared by Dr. Bhashyam S. Iyengar at The University of Arizona and were tested in the same lab as the compounds prepared by total synthesis. The data for these compounds is presented in Table 2.

Table 1: Activities of substituted mitosenes vs. P388 leukemia

COMPOUND	MAXIMUM %T/C	O.D. mg/kg	M.E.D. mg/kg	T.R.
<u>18</u>	128(183)	51.2	51.2	1
<u>19</u>	113(181)	6.4	-	-
<u>17</u>	165(229)	6.4	0.8	8
<u>20</u>	163(238)	6.4	0.4	16
<u>21</u>	167(228)	6.4	1.6	4
<u>22</u>	150(244)	3.2	1.6	2
<u>23</u>	111(183)	25.6	-	-
<u>24</u>	111(224)	6.4	-	-
<u>25</u>	128(211)	6.4	6.4	1
<u>26</u>	122(224)	6.4	-	-
<u>27</u>	144(183)	51.2	0.8	64
<u>29</u>	106(233)	25.6	-	-
<u>32</u>	150(181)	6.4	1.6	4
<u>36</u>	139(222)	12.8	12.8	1

Table 2: Activities of aziridinomitosenes vs. P388 leukemia



X	R	MAX. %T/C	O.D. mg/kg	M.E.D. mg/kg	T.R.
CH ₃ O	CH ₃	213 (181)	3.2	0.8	4
CH ₃ O	H	167 (250)	25.6	12.8	2
HC≡CCH ₂ NH	H	172 (228)	64.0	16.0	4
	H	183 (228)	64.0	8.0	8
	H	219 (244)	51.2	1.6	32
		239 (217)	25.6	1.6	16
	H	206 (217)	51.2	1.6	32
		CH ₃	222 (244)	6.4	0.2

Discussion

In evaluating the antineoplastic activity shown by the substituted mitosenes one can see that a leaving group is required at the 1-position in order to see appreciable activity. Thus compound 18 with no leaving group has only minimal effectiveness at high doses and compound 19 with a poor leaving group (hydroxyl) showed no acceptable levels of activity at any dosage. When a leaving group is present, as in compounds 17 and 20-27, considerable antineoplastic activity can be seen as shown by the T/C values for 17, 20, 21, 22, 25 and 27, all of which clearly exceed the minimal accepted level of 125. None of these compounds, however, are as effective as mitomycin C against P388 leukemia.

It is interesting to note that the compounds with the lowest T/C values, 23 and 24, were the most lipophilic of the group, supporting the premise that hydrophilic agents of the mitomycin family are more active than lipophilic agents when tested against leukemias. Also of interest is the observation that the leaving ability of the 1-substituent does not strictly parallel activity. For example the increasing order of leaving ability $\text{OH} < \text{OCOCH}_3 < \text{OCOCH}_2\text{Cl}$ is not followed in biological activity where $\text{OCOCH}_2\text{Cl} < \text{OH} < \text{OCOCH}_3$. Thus it is only safe to say that a reasonably good leaving group is required and that more subtle factors influence which leaving group is best.

Substitution of the 7-methoxyl group with a 7-(1-aziridinyl) moiety seems to be of little value in terms of increasing selectivity against tumor cells. The two compounds with 7-(1-aziridinyl) substituents, 26 and 27, do not show significantly better T/C values than their 7-methoxyl counterparts, 17 and 20, respectively. Compound 27 does seem to be less toxic than 20, however, since 20 showed a toxic response at doses above 12.8 mg/kg whereas 27 exhibited maximal effectiveness in that dosage range.

Some miscellaneous synthetic compounds were also tested even though they were not original targets for analogs. The first of these, 29 was tested in hope that the 8-nitro-pyrrolo[1,2-a]indole would be metabolically reduced to the corresponding amino derivative, 30. It was theorized that 30 would be an alkylating agent much like the hydroquinone moieties associated with mitomycin alkylation. Indeed, chemical reduction did produce a very unstable species but in vivo the compound does not appear to be reduced effectively since treated animals had essentially the same MST as untreated animals and no toxic response was seen. Some recent work by Sartorelli's group at Yale University has shown considerable antineoplastic activity for nitrobenzyl halides and carbamates using an assay of hypoxic EMT6 tumor cells in culture⁷⁶. Activity of these compounds is diminished when the tumor cells are thoroughly oxygenated. Considering 29 to be an analog of a nitrobenzyl carbamate,

perhaps P388 is a bad choice for detecting antineoplastic activity in this compound since the experiment involves well oxygenated cancer cells.

The established antibacterial activity of the oxime, 32⁶⁸, led to its testing against P388 leukemia as a second miscellaneous compound. In fact, the compound did show antineoplastic activity on a par with some of the other 1-substituted analogs. This result is somewhat difficult to explain since an oxime would not be a viable leaving group at the 1-position. Perhaps the observed activity can be rationalized by a metabolic reduction of the oxime to a 1-hydroxylamino derivative which would then have the necessary leaving group at the 1-position. Otherwise the activity might solely be due to a mechanism based on generation of peroxides from a cyclic reduction-oxidation of the quinone.

The last miscellaneous compound tested was the 10-chloromitosenone derivative, 36. Based on the extreme ease of hydrolysis of the chloro substituent, a low level of activity was expected since hydrolysis would create a poor leaving group (hydroxyl) at the 1-position and thus make bifunctional alkylation difficult. Testing showed the compound to be active (T/C = 139) but it is inferior to a hydrolytically more stable compound like 21 (T/C = 167).

One glance at the data for semisynthetic aziridinomitosenes (Table 3) reveals that they have much better

antineoplastic activity within the P388 system. T/C values for these analogs are all considerably higher than the 1-substituted analogs. In a few cases the maximum effectiveness actually exceeded that of mitomycin C. It is a curious fact that while maintaining comparable levels of activity the aziridinomitosenes are in general less potent than mitomycin C. This is in contrast to the 1-substituted analogs which have potencies close to that of mitomycin C (O.D. = 3.2 mg/kg in most of the experiments). The marked increase in level of antineoplastic activity for aziridinomitosenes relative to 1-substituted mitosenes most assuredly is due to the differences between aziridine opening and the leaving of an ester at the 1-position. The aziridine has special qualities that are difficult to reproduce in other leaving groups. To begin with it is slightly basic and protonation enhances cleavage of the 1C-N bond. Second, opening of the aziridine ring relieves strain associated with a three member ring making it a very good leaving group. Third, the semisynthetic compounds retain the stereochemistry of the natural molecules, a fact that may be of some importance at chiral sites of alkylation. The synthetic derivatives are of course a racemic mixture at the 1-position.

Compound 25 was synthesized in an attempt to mimic the protonatability of an aziridine as a leaving group. The pKa of a nicotinate ester⁷⁷ is reasonably close to that of

the mitomycin C aziridine⁸ (3.1 and 4.0 respectively). Since the nicotinate nitrogen is in conjugation with the carbonyl, one would expect protonation to enhance leaving ability by an inductive effect. Chemically this appears to be the case because the nicotinate ester of 25 is hydrolysed rapidly by aqueous acid. Unfortunately, although active, the behavior of 25 chemically did not predict superior antineoplastic activity. The effect of ring strain would probably be very difficult to duplicate with an ester-like leaving group but it might be worthwhile to synthesize an analog that could be resolved into optically pure isomers of the 1-position. That way each stereoisomer could be screened separately to determine the importance of stereochemistry at the 1-position.

An additional observation not apparent from the data presented in Table 2 is that a great many of the compounds exhibited toxic responses at higher doses. Compounds 19, 20, 22, 24, 26 and 32 were all toxic above 12.8 mg/kg. Clearly many of the 1-substituted and 1,7-disubstituted mitosenes prepared are potent and highly active cytotoxins. The problem seems to be that there is little selectivity for the leukemia cells over normally dividing cells. Again, it would seem most appropriate to blame a good part of this lack of selectivity on the substitution of an ester-like leaving group for the aziridine ring. Quinone reduction potential could also be causing the low selectivity but this

seems unlikely since the aziridinomitosenes with identical 7-substituents are so active. One would expect similar reduction potentials for compounds with the same chromophore. The other factor which could be involved here is lipophilicity. In general, the synthetic mitosenes have very limited water solubility as they are more lipophilic than mitosanes or aziridinomitosenes. This would tend to favor distribution into tissues such as bone marrow, liver and kidney resulting in lethal toxicities. In this respect, derivatives that would give higher water solubility might give better efficacy against leukemia whereas the derivatives made might show better activity against solid tumors. One way to increase water solubility would be to change the 7-methoxyl group to an amino or ethanolamino group. Another way would be to create more water soluble leaving groups at the 1-position such as esterification with glycine, alanine or threonine. Perhaps these types of modifications are areas that are worthy of future investigation.

In summary, the synthetic mitosenes prepared indicate a number of important structure-activity relationships relevant to both mitosanes and mitosenes. First, two active leaving groups are required for good antineoplastic activity. This observation lends support to the proposed mechanism of bioreductive alkylation with bifunctional alkylation of DNA being an important contributor to antineoplastic action. Second, the properties of the aziridine ring are intimately

associated with tumor cell selectivity and are very hard to duplicate in other leaving groups at the 1-position. This would seem to indicate that monofunctional, acid catalysed alkylation is also an important process in cancer cell kill. Third, good water solubility is required for activity against leukemia. As indicated by the P388 test results, the synthetic mitosenes prepared are potent and highly active cell toxins but their activity specifically against cancer cells is not good enough to warrant further serious consideration when compared to other mitomycin analogs such as aziridinomitosenes. Additional testing for selected analogs against L1210 leukemia and against EMT6 tumor cells under hypoxic conditions will be performed to verify the P388 results. This work will be reported elsewhere.

EXPERIMENTAL

General

Melting points were determined on a Laboratory Instruments Mel-Temp apparatus and are uncorrected. IR spectra were taken on a Beckman IR-33 spectrometer with samples prepared as potassium bromide pellets. Absorptions are reported in cm^{-1} . NMR spectra were taken on a Varian EM-360L 60 MHz spectrometer and absorptions are reported as ppm downfield from TMS. Mass spectral data were obtained on a Varian 311A instrument courtesy of Mr. Peter F. Baker and Dr. Karl H. Schram from the Department of Pharmaceutical Sciences, University of Arizona. Ionizing voltage was 70 e.v. and the source temperature was 250°C . Samples were introduced by direct probe inlet. Reported are the molecular ion followed by the most intense ion unless otherwise indicated. Elemental analyses were performed either by the University of Arizona Analytical Center or Galbraith Laboratories, Inc. Thin layer chromatography (TLC) was carried out using commercially prepared 2 mm layer of Silica Gel 60 on plastic sheets. The phrase "chromatography on silica gel" denotes the use of Silica Gel 60 (70-270 mesh) with gravity elution. The acronym "LPC" (low pressure chromatography) indicates the use of MN-Silica Gel P, intended for preparation of TLC plates, packed in a

column and elution accomplished at 2-3 psi (pressure generated by an argon cylinder). Adsorbents for these three forms of chromatography were purchased from Brinkmann Instruments, Inc. Preparative scale high performance liquid chromatography (HPLC) was carried out on a Waters Associates Preparative 500 instrument using a single Waters PrepPAK-500 Silica column. Semi-preparative scale HPLC was performed using a Spectra-Physics model 3500 liquid chromatograph and a Whatman Partisil M9 10/50 column. Reference samples were available through the previous work of Dennis L. Fost³²⁻³⁴ and James Mott³⁵.

2,5-Dimethyl-4-nitrophenol (2)

A mechanically stirred mixture of 2,5-dimethylphenol (400 g, 3.28 mol), glacial acetic acid (980 mL) and sulfuric acid (130 mL) was cooled to 5°C on an ice bath. To this mixture was slowly added a solution of sodium nitrite in water (230 g, 3.33 mol in 660 mL) in portions so that the temperature was maintained below 10°C. During the addition the reaction mixture was flushed with nitrogen. Stirring was continued 20 min. past the last addition of sodium nitrite solution. The resulting mixture was rinsed from the flask with cold water (2 L) and filtered to give the crude nitroso product as an orange-brown solid. The partially dried nitroso derivative was added in 10 portions over 24 h. to 52.5% nitric acid (1.4 L) in a large beaker

equipped with mechanical stirring and good ventilation. Nitrogen oxides formed as by-products were visible escaping the reaction mixture as the oxidation took place. Successive portions of the nitroso derivative were not added until no brown gas could be seen as a result of the previous addition. Stirring was continued two hours past the last addition. The product was then collected by filtration and rinsed well with cold water. The crude nitro product, 2, was isolated as a tan solid: MP 110-114°C. It was used directly in the next step. Recrystallization of a small portion from benzene gave crystalline material: MP 121-123°C; IR 3375, 1620, 1575, 1480, 1315; NMR (CDCl₃) 8.0 (s, 1H), 6.4 (s, 1H), 2.6 (s, 3H), 2.3 (s, 3H); TLC (methylene chloride-methanol, 95:5) of this preparation was identical with an authentic sample.

2,5-Dimethyl-4-nitroanisole (3)

A mechanically stirred mixture of the partially dried nitro derivative prepared above and water (850 mL) was heated to 40°C and treated alternately, in small portions, first with 9.5 M sodium hydroxide (600 mL, 5.7 mol) and then with dimethyl sulfate (500 mL, 2.98 mol). Alternating portions were added at a rate that maintained the temperature below 45°C and stirring was continued 20 min. past the last addition. The resultant brown slurry was cooled to 10°C, filtered and the solid rinsed well with cold water until

most of the brown impurities were removed, leaving the anisole derivative, 3, as a dirty white solid. Two recrystallizations from methanol gave 3 (340 g, 57% from 1) as off-white crystals: MP 87-89°C; IR lacks 3375 OH absorption of 2; NMR (CDCl₃) 7.9 (s, 1H), 6.8 (s, 1H), 3.9 (s, 3H), 2.6 (s, 3H), 2.2 (s, 3H); TLC (chloroform) of this preparation was identical with an authentic sample.

5-Methoxy-4-methyl-2-nitrophenylpyruvic Acid (4)

Potassium t-butoxide was prepared by reacting potassium metal (15.8 g, 0.4 mol) with refluxing t-butanol (320 mL, freshly dried over calcium hydride and distilled). Excess t-butanol was evaporated under reduced pressure and the resulting solid was suspended in benzene (200 mL, freshly dried over sodium and distilled). This mixture was evaporated at reduced pressure and the benzene addition and removal was repeated a second time. Finally, the resulting white solid was suspended in dry benzene (320 mL). A mechanically stirred solution of 3 (73 g, 0.4 mol) in dry benzene (900 mL) was treated with the slurry resulting from the addition of diethyl oxalate (64 mL, 0.47 mol, freshly distilled) to the potassium t-butoxide suspension. The reaction mixture was heated at reflux under nitrogen for 12 h. and the resulting maroon paste was allowed to sit at R.T. for 24 h. The maroon solid was collected by filtration, washed well with ether and air dried. It was then boiled in

3.2% aqueous sodium bicarbonate (1.5 L) for 30 min., cooled to 15°C, extracted with ether and acidified with 37% hydrochloric acid to precipitate the pyruvic acid derivative, 4. The product, collected by filtration, was further purified by suspension in and filtration from benzene. Drying at reduced pressure gave 4 (90 g, 89%) as a tan solid: MP 163-165°C; IR 3050, 1745, 1710; NMR (CDCl₃ + DMSO-d₆) 7.80 (d, 1H), 6.90 (d, 1H), 4.51 (s, 2H), 3.92 (s, 3H), 2.20 (s, 3H); TLC (chloroform-methanol, 7:3) of this preparation was identical to an authentic sample.

5-Methoxy-6-methyl-2-indolecarboxylic Acid (5)

A mechanically stirred solution of ferrous sulfate heptahydrate (483 g, 1.74 mol) and water (550 mL) was heated to 85°C and treated slowly with a solution of the crude pyruvic acid derivative, 4 (60.3 g, 0.24 mol), in 11.5% ammonium hydroxide (500 mL). After the addition was complete the mixture was stirred at 85-100°C for 1 h. and cooled to R.T. Centrifugation separated a black solid from a brown solution. The solution was decanted, filtered and acidified with 37% hydrochloric acid to precipitate 5. The black solid was then successively suspended in 11.5% ammonium hydroxide and recentrifuged until the filtered supernatant yielded no product upon acidification. The combined indolecarboxylic acid, 5, was collected by filtration, washed well with water and dried at reduced pressure to give

32.3 g (66%) as a tan solid: MP 238-241°C (decomposes); IR 3390, 3100-2900, 1665, 1520; TLC (chloroform-methanol, 4:1) of this material was identical with an authentic sample.

Methyl 5-methoxy-6-methyl-2-indolecarboxylate (6)

A. A mixture of 5 (33 g, 0.16 mol), dry methanol (900 mL) and conc. sulfuric acid (14 mL) was stirred first at reflux for 4 h. and then at R.T. overnight. The excess methanol was evaporated at reduced pressure and the resulting solid was dissolved in chloroform (800 mL). Extraction with 5% aqueous sodium bicarbonate (4 X 250 mL) removed some starting material which was recovered by acidification of the aqueous fractions and filtration: 4.6 g (14%) of 5 was recovered. The organic phase was dried over magnesium sulfate and evaporated to a concentrated solution which was chromatographed on silica gel with chloroform. Collection of the major, pale colored band gave 6 (22.5 g, 64%) as a white, crystalline solid: MP 144-146°C; IR 3340, 1687, lacks COOH absorption of 5; NMR (CDCl₃) 7.15 (s, 2H), 6.90 (s, 1H), 3.92 (s, 3H), 3.85 (s, 3H), 2.30 (s, 3H); TLC (methylene chloride-hexane, 3:1) of this material was identical with an authentic sample.

B. A solution of 5 (65 g, 0.32 mol) in dry methanol (1.8 L) was bubbled with a slow stream of anhydrous hydrogen chloride at reflux for 24 h. It was then flushed with -

nitrogen and cooled to R.T. Dilution with 5% sodium bicarbonate saturated with sodium chloride (2.5 L) precipitated the product, 6, which was collected by filtration, washed well with water and dried at reduced pressure to give 53 g (76%) as a tan solid. Recrystallization of several combined batches from methanol gave material of identical purity and spectral properties to that produced by method A.

Methyl 2,3-dihydro-7-methoxy-6-methyl-1-oxo-1H-pyrrolo[1,2-a]indole-2-carboxylate (7)

Fresh potassium t-butoxide was prepared as described above from potassium metal (4.4 g, 112 mmol) and t-butanol (100 mL). It was suspended in dry benzene (250 mL) and then added to a solution of 6 (22.5 g, 103 mmol) in dry benzene (750 mL). This mixture was stirred mechanically a few min. before adding methyl acrylate (9.2 mL, 103 mmol, freshly distilled). The mixture was then heated to reflux for 18 h. and, after cooling, it was diluted with water, neutralized with 1 N hydrochloric acid and extracted with chloroform. The organic fraction was evaporated under a vacuum and the residue was suspended in cold methanol. The undissolved solid was then quickly collected by filtration and air dried to give 7 (20 g, 71%) as an off-white solid that was sufficiently pure for the next step: MP 180-181°C; IR 1700, 1640, 1525, 1440; NMR (CDCl₃) 7.21 (s, 1H), 6.96 (s, 1H), 6.93 (s, 1H), 4.50 (m, 3H), 3.86 (s, 3H), 3.82 (s, 3H),

2.35 (s, 3H); TLC (chloroform) of this material was identical with an authentic sample.

2,3-Dihydro-7-methoxy-6-methyl-1H-pyrrolo-
[1,2-a]indol-1-one (8)

A mechanically stirred mixture of 95% acetic acid (800 mL) and 7 (37.2 g, 137 mmol) was heated at reflux for 18 h. After cooling, the mixture was diluted with water (800 mL), cooled back to R.T., filtered and the solid rinsed well with water to give 8 (24.3 g) as a brown crystalline solid. The combined mother liquor and washings were chilled overnight to precipitate an additional 2 g of product (89% total yield). This material was purified in batches by chromatography on silica gel with chloroform to give pale yellow crystals: MP 206-208°C; IR 1700, 1525, 1390; NMR (CDCl₃) 7.15 (s, 1H), 7.00 (s, 1H), 6.85 (s, 1H), 4.30 (t, 2H), 3.91 (s, 3H), 3.15 (t, 2H), 2.35 (s, 3H); TLC (chloroform) of this material was identical with an authentic sample.

2,3-Dihydro-1-hydroxy-7-methoxy-6-methyl-
1H-pyrrolo[1,2-a]indole (9)

A magnetically stirred mixture of 8 (3.0 g, 13.8 mmol), sodium borohydride (2.33 g, 61 mmol) and ethanol (400 mL) was allowed to react at room temperature until the initial suspension became a clear solution (4-5 h.) Excess ethanol was evaporated at reduced pressure to give a solid

that was dissolved in ether and stirred vigorously with an equal volume of water for 30 min. The organic fraction was then separated, dried over magnesium sulfate and evaporated to give 9 (2.7 g, 90%) as a white solid which was used immediately in the next step as it is not stable: IR 3500-3200, 2900, lacks 1700 ketone absorption of 8; NMR (CDCl₃) 6.95 (s, 2H), 6.20 (s, 1H), 5.25 (d of d, 1H), 4.00 (m, 2H), 3.81 (s, 3H), 2.80 (m, 2H), 2.31 (s, 3H).

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole (10)

The tricyclic alcohol prepared above (2.7 g, 12.4 mmol) was dissolved in pyridine (60 mL) and treated with acetic anhydride (40 mL). The mixture was stirred 16 h. at R.T., poured into ice water (500 mL) and stirred 15 min. before collecting the resulting precipitate by filtration. The solid was dissolved in methylene chloride, washed well with water, dried over magnesium sulfate and evaporated at reduced pressure to give 10 (2.9 g, 90%) as a white solid: MP 131-132°C; IR 1727, lacks 3500-3200 OH absorption of 9; NMR (CDCl₃) 7.01 (s, 1H), 6.97 (s, 1H), 6.30 (s, 1H), 6.10 (d of d, 1H), 4.12 (m, 2H), 3.85 (s, 3H), 2.81 (m, 2H), 2.35 (s, 3H), 2.05 (s, 3H).

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde (11)

DMF was dried over potassium hydroxide and distilled immediately prior to use in both procedures.

A. At -5°C phosphorous oxychloride (0.68 mL, 7.43 mmol) was added dropwise to DMF (10 mL) and the mixture stirred about 10 min. A solution of 10 (1.7 g, 6.56 mmol) in DMF (75 mL) was added over 1 h. and the reaction was stirred an additional hour at R.T. The mixture was then added dropwise to saturated aqueous sodium acetate (400 mL) at -10°C and stirred 4 h. while gradually letting the temperature rise to R.T. The resulting solid was filtered, rinsed well with water, dissolved in chloroform and dried over magnesium sulfate to give crude 11 as a tan solid upon evaporation. This was suspended in ethyl acetate at 40°C , chilled to -10°C and filtered to give 11 (1.35 g, 72%) as a nearly white solid: MP $166-168^{\circ}\text{C}$; IR 3000-2900, 2825, 1730, 1640; NMR (CDCl_3) 10.02 (s, 1H), 7.70 (s, 1H), 7.05 (s, 1H), 6.45 (d of d, 1H), 4.21 (m, 2H), 3.93 (s, 3H), 2.85 (m, 2H), 2.35 (s, 3H), 2.10 (s, 3H); TLC (chloroform) of this preparation was identical to an authentic sample.

B. A mixture of methylene chloride (5 mL) and DMF (2 mL, 25.8 mmol) was treated at 5°C with phosphorous oxychloride (1.2 mL, 13.1 mmol, freshly distilled) and stirred 15 min. under argon. This mixture was then treated with a solution composed of 10 (3.0 g, 11.6 mmol), dry pyridine

(5 mL, 62.1 mmol) and methylene chloride (55 mL). The reaction was stirred at 5°C for 50 min., treated with cold saturated sodium acetate (225 mL) and stirred vigorously 4 h. The orange organic phase was separated from the red aqueous phase and washed with 1.5 N hydrochloric acid (3 X 200 mL) followed by water (1 X 200 mL). After drying over magnesium sulfate, the solvent was evaporated to give 11 (3.24 g, 97.5%) as an off-white solid which had the same spectral characteristics as that produced by method A.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde (12)

A mixture of 11 (3.0 g, 11.6 mmol) and glacial acetic acid (50 mL) was treated with 90% nitric acid (3.0 mL) and stirred 30 min at R.T. It was then poured into ice water (600 mL) and stirred a few minutes before the precipitate was collected by filtration, washed well with water, dissolved in chloroform, extracted with water, dried over magnesium sulfate and evaporated at reduced pressure. The crude nitro compound, 12, was purified by chromatography on silica gel with chloroform to give 3.3 g (85%) as a pale yellow solid: MP 176-178°C; IR 1750, 1665, 1535, 1380; NMR (CDCl₃) 9.73 (s, 1H), 7.20 (s, 1H), 6.33 (d of d, 1H), 4.20 (t, 2H), 3.77 (s, 3H), 3.00 (m, 2H), 2.34 (s, 3H), 2.00 (s, 3H); TLC (chloroform) was identical with an authentic sample.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-5,8-dioxo-1H-pyrrolo[1,2-a]indole-9-carboxaldehyde (14)

Finely ground 12 (1.0 g, 3.0 mmol) was suspended in ethanol (250 mL) and treated with tin metal (3.2 g, 30 mesh granules) and 3 N hydrochloric acid (68 mL). The mixture was stirred vigorously for 70 min. and the resulting clear solution was decanted from excess tin. Concentrated aqueous sodium bicarbonate was added dropwise until a white precipitate formed and the mixture was then extracted with chloroform to remove a yellow organic fraction which was washed with water, dried over magnesium sulfate and evaporated to give the amine, 13, as a thick yellow oil which was used without purification in the next step: IR 3440, 3320, 2940, 1745, 1645; NMR (CDCl₃) 9.60 (s, 1H), 6.25 (m, 2H), 5.67 (broad, 2H), 4.00 (t, 2H), 3.73 (s, 3H), 2.80 (m, 2H), 2.33 (s, 3H), 2.05 (s, 3H).

The amine from above was dissolved in acetone (180 mL) and treated with a solution of potassium nitrosodisulfonate (4.5 g) in 0.3 M, pH 6.0 phosphate buffer (180 mL). The reaction mixture was stirred 2 h., diluted with water and extracted with methylene chloride to give an orange organic fraction and a purple aqueous fraction. The organic fraction was washed with 5% sodium carbonate (2 X 200 mL) and water (1 X 200 mL), then dried over magnesium sulfate and concentrated. The residue was immediately purified on silica gel by eluting with chloroform to give an orange oil

that precipitated upon evaporation from acetone-hexane at reduced pressure to give 14 (777 mg, 81.7%) as a bright orange solid: MP 139-140^oC; IR 3020-2860, 1740, 1680, 1660, 1640; NMR (CDCl₃) 10.33 (s, 1H), 6.32 (d of d, 1H), 4.29 (m, 2H), 4.06 (s, 3H), 2.90 (m, 2H), 2.10 (s, 3H), 1.99 (s, 3H). This material was identical in spectral and TLC (chloroform) properties to that produced by established procedures but its melting point is higher (Lit.³³ MP 126-127). Occasionally the product required additional purification in order to obtain a solid. This could be accomplished by preparative HPLC, eluting with methylene chloride-acetone (95:5) at 250 mL/min. The impurity was a small quantity of an unidentified yellow oil with a slightly longer retention time.

1-Acetoxy-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-5,8-dione (15)

A. A solution of ethanol (40 mL, dried at reflux over magnesium and distilled), tetrahydrofuran (20 mL, dried over lithium aluminum hydride and distilled) and glacial acetic acid (0.05 mL) was repeatedly evacuated and repressurized with argon to remove all oxygen. It was then treated with sodium borohydride (350 mg, 9.2 mmol) and after bubbling stopped the system was again flushed with argon. Finally, solid 14 (350 mg, 1.1 mmol) was added, the system was flushed and stirred under a stream of argon until

the orange color faded to nearly a colorless solution (approx. 10 min.). During the reaction, acetone (10 mL) was bubbled with argon to remove air. This acetone was added and the reaction mixture stirred two minutes before adding dropwise 1 N ferric chloride in 0.1 N aqueous hydrochloric acid (10 mL). The mixture was diluted with water and methylene chloride, the organic phase was separated and the aqueous layer washed with methylene chloride until no orange color was extracted. The combined organic fractions were washed with water, dried over magnesium sulfate, concentrated at reduced pressure and chromatographed on silica gel with chloroform. Collection of the major orange band and evaporation of solvent gave 15 (225 mg, 64.2%) as an orange solid: MP 112-113°C; IR 3420, 1730, 1640; NMR (CDCl₃) 6.01 (d of d, 1H), 4.67 (s, 2H), 4.30 (t, 2H), 4.00 (s, 3H), 2.75 (m, 2H), 2.09 (s, 3H), 1.97 (s, 3H). TLC (methylene chloride-acetone, 95:5) showed a minor red-orange spot just above the major orange spot. Purification of two or three combined batches by preparative HPLC, eluting with methylene chloride-acetone (95:5) at 250 mL/min., easily separated this minor component which has the spectral characteristics of 16: NMR (CDCl₃) 4.58 (s, 2H), 4.20 (m, 2H), 4.00 (s, 3H), 2.65 (m, 4H), 2.00 (s, 3H); MS 261, 246. This separation was not routinely carried out since after further

steps in the synthesis the mixture of 18 and 19 was easily separated by gravity chromatography.

B. A solution of 14 (200 mg, 0.63 mmol) in tetrahydrofuran (7.5 mL, freshly dried over lithium aluminum hydride and distilled) was chilled under nitrogen to -10°C and treated with 0.5 M 9-borabicyclo[3.3.1]nonane in tetrahydrofuran (2.6 mL, 1.3 mmol). The reaction was stirred under nitrogen as it slowly warmed to R.T. over 20 min. It was then returned to the ice bath and treated with 30% hydrogen peroxide (10 mL, 1 mL at a time). After stirring for several minutes the mixture was diluted with water and extracted with methylene chloride. The organic fraction was washed with water, dried over magnesium sulfate and concentrated at reduced pressure to a dirty orange residue. It was then immediately purified by chromatography on silica gel after first adsorbing on a small quantity of silica gel from acetone solution. The elution solvent used for chromatography was methylene chloride-acetone (95:5). The major orange band was collected and the solvent was removed at reduced pressure to give 15 (144 mg, 71.5%) as an orange solid. This material was identical in spectral and TLC (methylene chloride-acetone 9:1) properties to that prepared by method A except that no 16 was visible on the TLC chromatogram.

1-Acetoxy-7-methoxymitosene (17)

A solution of 15 (151 mg, 0.47 mmol), methylene chloride (30 mL) and pyridine (3 mL) was treated at 5°C with phenyl chloroformate (1.5 mL, 6.3 mmol). The mixture was stirred overnight at R.T., chilled on an ice bath and diluted with water. The organic phase was separated, washed with 3 N hydrochloric acid (3 X 75 mL) and water (1 X 100 mL), dried over magnesium sulfate and evaporated to an oil which slowly crystallizes. Chromatography on silica gel with toluene eluted some diphenyl carbonate. The solvent was changed to chloroform to elute the phenylcarbonate derivative as a single orange band which evaporated to an orange oil: NMR (CDCl₃) 7.28 (m, 5H), 6.17 (d of d, 1H), 5.47 (s, 2H), 4.33 (t, 2H), 4.02 (s, 3H), 2.80 (m, 2H), 2.07 (s, 3H), 1.95 (s, 3H).

The material from above was dissolved in 30 mL methylene chloride, chilled on a dry ice-acetone bath and bubbled with anhydrous ammonia until the volume reached about 45 mL. The reaction was then allowed to rise to R.T. and was stirred until no ammonia could be seen escaping. It was warmed slightly to expell the last traces of ammonia, diluted with methylene chloride to dissolve some precipitated product and washed many times with water to remove phenol. The organic fraction was dried over magnesium sulfate, concentrated at reduced pressure and purified by

LPC using chloroform-acetone (9:1) as eluent. Collection of the major orange band gave 17 (163 mg, 91%) as an orange solid upon evaporation: MP 204-205°C (decomposes); IR 3460, 3320, 1730, 1705, 1655; NMR (CDCl₃) 6.28 (d of d, 1H), 5.27 (s, 2H), 4.80 (broad, 2H), 4.28 (t, 2H), 4.01 (s, 3H), 2.72 (m, 2H), 2.07 (s, 3H), 1.96 (s, 3H); MS 362, 259; TLC (methylene chloride-acetone, 4:1) was identical to an authentic sample.

1-Hydroxy-7-methoxymitosene (19) and
7-Methoxymitosene (18)

A. A solution of 17 (150 mg, 0.41 mmol) in methanol (175 mL) was treated with 30% ammonium hydroxide (5 mL) and stirred 10 h. at R.T. The methanol was removed at reduced pressure and the residue was dissolved in methylene chloride for purification on silica gel with methylene chloride-acetone (4:1) as eluent. A small orange band eluted ahead of the major orange band (not seen when the 9-BBN method was used earlier in the synthesis to prepare 15). Collection of the former and evaporation of solvent gave 18 (5 mg, 6% of the product) as an orange-red solid: IR 3410, 3300, 1685, 1640; NMR (CDCl₃) 5.22 (s, 2H), 4.65 (broad, 2H), 4.18 (t, 2H), 4.00 (s, 3H), 2.70 (m, 4H), 1.95 (s, 3H); TLC (methylene chloride-acetone, 6:1) was identical with an authentic sample. Collection of several batches and recrystallization from ethanol gave orange needles: MP 205-206°C. The major

orange band gave 19 (103 mg, 77.7%) as an orange solid upon evaporation of solvent: MP 189-190°C; IR 3400, 1685, 1640; NMR (DMSO-d₆) 6.10 (broad, 2H), 5.12 (s, 3H), 4.19 (t, 2H), 3.93 (s, 3H), 3.20 (s, H₂O), 2.50 (m, 2H), 1.90 (s, 3H); TLC (methylene chloride-acetone, 4:1) was identical with an authentic sample.

B. A solution of 17 (200 mg, 0.55 mmol) in methanol (250 mL) was treated with 1.0 N potassium hydroxide (1.6 mL) and stirred 3 h. at R.T. The solution was then acidified with 1.0 N hydrochloric acid (1.5 mL) and the methanol was evaporated at reduced pressure. The residue was adsorbed on silica gel by evaporation from methylene chloride and then it was purified by chromatography on silica gel, eluting with methylene chloride-acetone (4:1). Collection of the major orange band and evaporation of solvent gave 19 (159 mg, 89.9%) as an orange solid that was identical to that produced by method A.

1-Hydroxy-7-methoxymitosene Carbamate (20)

A solution of 19 (110 mg, 0.36 mmol) in pyridine (25 mL) and methylene chloride (50 mL) was chilled to 0°C, treated with phenyl chloroformate (2.5 mL, 10.5 mmol) and stirred overnight at R.T. The mixture was chilled on an ice bath and diluted with water. It was then extracted with methylene chloride and the organic fraction was washed with 3 N hydrochloric acid (4 X 50 mL) and water (1 X 100 mL),

dried over magnesium sulfate and evaporated at reduced pressure. Chromatography of the resulting oil on silica gel with toluene removed some diphenyl carbonate. Elution with chloroform then gave one major orange band and several lesser orange bands. Collection of the major band and evaporation of solvent gave the phenylcarbonate derivative as an orange oil which was difficult to dry to a solid: NMR (CDCl_3) 7.25 (m, 5H), 6.3 (d of d, 1H), 5.33 (s, 2H), 4.89 (broad, 2H), 4.39 (t, 2H), 4.03 (s, 3H), 2.85 (m, 2H), 1.97 (s, 3H).

The oil from above was dissolved in 50 mL methylene chloride and chilled on a dry ice-acetone bath while anhydrous ammonia was bubbled into it. When the volume reached about 65 mL the bubbling was stopped, the flask was removed from the bath and the contents were slowly warmed to R.T. over 3 h. while stirring. After the ammonia had boiled away the resulting mixture was evaporated to a sticky solid which was adsorbed on a small quantity of silica gel by stirring in methylene chloride and evaporating to dryness at reduced pressure. The adsorbed product was purified by chromatography on silica gel, eluting with methylene chloride-methanol (95:5) to obtain 20 (60 mg, 48.1%) as a yellow solid upon evaporation of solvent from the major yellow band: MP 200°C (decomposes; IR 3460-3300, 1680, 1640; NMR (CDCl_3 + $\text{DMSO}-d_6$) 6.30 (broad, 2.5H), 6.07 (broad, 2.5H), 5.13 (s,

2H), 4.20 (m, 2H), 3.97 (s, 3H), 3.30 (s, H₂O), 2.55 (m, DMSO), 1.90 (s, 3H); MS 363, 259.

Repeated attempts to obtain an elemental analysis gave results inconsistent with the molecular formula despite the overwhelming spectral evidence supporting the designated structure. Hence 20 was purified by semi-preparative scale HPLC as follows: A solution of 20 (7 mg) in DMF-methylene chloride (1:1, 3.5 mL) was chromatographed (approx. 0.4 mL per run) by eluting first with methylene chloride for 3 min. and then introducing a 0-5% linear gradient of methanol over 20 min. Elution rate was 5 mL/min. Some non-polar material eluted first, followed by DMF. Next to elute were a major yellow fraction and four minor components. Center cuts of the major component were collected for each run and combined. A portion (0.5 mL) was then injected and eluted as above to give a single sharp peak on the recorder chart, indicating pure 20. Evaporation of solvent gave 20 as a slightly sticky yellow solid. This was triturated with acetone and dried at R.T. under a vacuum for 48 h. Anal. calcd. for C₁₆H₁₇N₃O₇·(CH₃)₂CO: C 54.1, H 5.5, N 9.9; found: C 54.2, H 5.6, N 9.8.

1-Hydroxy-7-methoxymitosene Methylcarbamate (21)

A suspension of potassium carbonate (2 g) in methylene chloride was treated with methyl isocyanate (1 mL, 16.2 mmol) and stirred 5 min. before adding a solution of 19

(21 mg, 0.06 mmol) in methylene chloride (10 mL). The reaction was stoppered and stirred at R.T. for 15 h. It was then filtered and the potassium carbonate was rinsed with chloroform and ethyl acetate until colorless. The combined organic fraction was evaporated at reduced pressure and chromatographed on silica gel after first adsorbing on about 1 g of the gel. Elution with chloroform-ethanol (98:2) gave two yellow bands. The smaller eluted first and evaporated to a small quantity of an unidentified oil. The second, larger band gave 21 (12.8 mg, 51.7%) of the methylcarbamate as a bright yellow solid: MP 225°C (decomposes); IR 3460, 3300, 1680, 1640; NMR (CDCl₃ + DMSO-d₆) 6.20 (m, 1H), 5.15 (s, 2H), 4.92 (broad, 2H), 4.73 (broad, 1H), 4.20 (t, 2H), 3.91 (s, 3H), 2.85-2.50 (m, 5H), 1.75 (s, 3H); MS 377, 259; Anal. calcd. for C₁₇H₁₉N₃O₇: C 54.11, H 5.07, N 11.14; found: C 54.43, H 5.23, N 11.05.

1-Hydroxy-7-methoxymitosene Methylcarbonate (22)

A solution of 19 (50 mg, 0.16 mmol), methylene chloride (20 mL) and pyridine (5 mL) was treated at 0°C with methyl chloroformate (1 mL, 12.9 mmol). The reaction was stirred at R.T. 24 h. and then diluted with water. Extraction with methylene chloride removed an orange organic fraction which was washed with 3 N hydrochloric acid (4 X 50 mL) and water (1 X 100 mL), dried over magnesium sulfate and evaporated. The concentrated residue was purified by

LPC, eluting with chloroform-acetone (95:5) to give an orange solid that was difficult to dry. Recrystallization from ethanol afforded 22 (27 mg, 45.7%) as a crystalline orange solid: MP 187-190°C; IR 3440, 3330, 3260, 1730, 1640; NMR (CDCl₃) 6.16 (d of d, 1H), 5.25 (s, 2H), 4.80 (broad, 2H), 4.27 (t, 2H), 3.93 (s, 3H), 3.75 (s, 3H), 2.71 (m, 2H), 1.85 (s, 3H); MS 378, 259; Anal. calcd. for C₁₇H₁₈N₂O₈: C 53.97, H 4.76, N 17.01; found: C 54.29, H 4.78, N 11.05.

1-Hydroxy-7-methoxymitosene Trimethylacetate (23)

A suspension of potassium carbonate (3 g) in ethyl acetate (2 mL) was treated with trimethylacetyl chloride (1.7 mL, 13.8 mmol) and stirred 5 min. before adding a solution of 19 (24.5 mg, 0.08 mmol) in ethyl acetate (10 mL). The reaction was stirred under argon 4 days and then diluted with water and ethyl acetate. The organic fraction was washed with water, dried over magnesium sulfate and evaporated at reduced pressure to give an orange solid suspended in a high boiling liquid (excess acid chloride). This mixture was dissolved in chloroform and purified by LPC, using chloroform as the eluent. Evaporation of solvent from the major band gave 23 (13.3 mg, 43.0%) as an orange solid: MP 238-239°C (decomposes); IR 3440, 3320, 3270, 1720, 1640, 1630; NMR (CDCl₃) 6.33 (m, 1H), 5.27 (s, 2H), 4.78 (broad, 2H), 4.30 (t, 2H), 4.00 (s, 3H), 2.75 (m, 2H), 1.97 (s, 3H),

1.20 (s, 9H); MS 404, 259; Anal. calcd. for $C_{20}H_{24}N_2O_7$:
C 59.39, H 5.98, N 6.93; found: C 59.32, H 6.37, N 6.82.

1-Hydroxy-7-methoxymitosene Chloroacetate (24)

A suspension of potassium carbonate (2 g) in ethyl acetate (2 mL) was treated with chloroacetyl chloride (0.7 mL, 8.8 mmol) and stirred a few minutes before adding a solution of 19 (16 mg, 0.05 mmol) in ethyl acetate (7 mL). The mixture was then stirred under nitrogen at R.T. for 12 h., diluted with water and ethyl acetate and the organic phase was washed several times with water. After drying over magnesium sulfate and evaporation of solvent, the residue was purified by LPC, eluting with methylene chloride-acetone (9:1) to give 24 (9.7 mg, 48.9%) as a bright orange solid: MP 135°C (decomposes); NMR ($CDCl_3$) 6.36 (d of d, 1H), 5.29 (s, 2H), 4.76 (broad, 2H), 4.32 (t, 2H), 4.10 (s, 2H), 4.08 (s, 3H), 2.75 (m, 2H), 1.97 (s, 3H); MS 396, 398 (3:1), 259; Anal. calcd. for $C_{17}H_{17}N_2O_7Cl$: C 51.46, H 4.32, N 7.06; found: C 51.56, H 4.26, N 7.06.

1-Hydroxy-7-methoxymitosene Nicotinate (25)

A solution of 19 (33.4 mg, 0.10 mmol), methylene chloride (6 mL) and dry pyridine (1 mL) was treated with nicotinyl chloride hydrochloride⁷¹ (150 mg, 1.09 mmol) and heated at reflux under nitrogen for 6 h. After cooling, water (0.25 mL) was added and the mixture was stirred

vigorously for 2 min. to hydrolyse excess acid chloride. It was then dried over magnesium sulfate and evaporated to an oil that smelled of pyridine. This oil was repeatedly diluted with toluene and evaporated at reduced pressure until no pyridine odor could be detected. The solid was triturated with methylene chloride in order to dissolve the orange product which was filtered from undissolved nicotinic acid by-product. The methylene chloride solution was then purified by LPC, eluting with methylene chloride-acetone-methanol (78:20:2) to give 25 (29.0 mg, 64.5%) as a yellow solid: MP 175-178°C (decomposes); IR 3460, 3370, 1732, 1700, 1660, 1640; NMR (CDCl₃ + DMSO-d₆) 9.12 (s, 1H), 8.79 (d of d, 1H), 8.31 (d of d, 1H), 7.46 (d of d, 1H), 6.44 (d of d, 1H), 5.98 (broad, 2H), 5.20 (s, 2H), 4.35 (t, 2H), 3.99 (s, 3H), 3.68 (s, H₂O), 2.85 (m, 2H), 2.51 (m, DMSO), 1.89 (s, 3H); MS 364 (M⁺-HOCONH₂), 259, 78 (C₅H₄N⁺); Anal. calcd. for C₂₁H₁₉N₃O₇: C 59.3, H 4.5, N 9.9; found: C 58.9, H 4.4, N 9.5.

7-(1-Aziridinyl)-1-hydroxymitosene Acetate (26)

A solution of 17 (38 mg, 0.12 mmol) in dry methanol (50 mL) was treated with ethylenimine⁷³ (2 mL) and stirred for 2.5 h. at R.T. The reaction mixture was then evaporated at reduced pressure and chromatographed on silica gel with methylene chloride-acetone (4:1). Collection of the major red band and evaporation of solvent gave 26 (26.8 mg,

68.7%) as a burgundy-colored solid: MP 204-205°C (decomposes); IR 3450-3200, 1730, 1640; NMR (CDCl₃) 6.30 (d of d, 1H), 5.28 (s, 2H), 4.86 (broad, 2H), 4.27 (t, 2H), 2.80 (s, 4H), 2.07 (s, 6H); MS 373, 270. Further purification was possible by recrystallization from ethanol: MP 208-210°C (decomposes); Anal. calcd. for C₁₈H₁₉N₃O₆: C 57.90, H 5.13, N 11.26; found: C 57.75, H 5.23, N 11.26.

A second, smaller red band that eluted from the column after 26 gave about 5 mg of a burgundy-colored solid that appeared by its NMR spectrum to be 7-(1-aziridinyl)-1-hydroxymitosene: NMR (CDCl₃ + DMSO-d₆) 6.13 (m, 1H), 5.90 (broad, 2H), 5.19 (d, 2H), 4.22 (t, 2H), 2.97 (s, H₂O), 2.26 (s, 4H), 1.99 (s, 3H).

7-(1-Aziridinyl)-1-hydroxymitosene Carbamate (27)

Compound 20 (33 mg) was dissolved in DMF (5 mL, dried over potassium hydroxide and distilled) and treated with ethylenimine⁷³ (1 mL). The solution changed from yellow to deep red in 20 min. but the mixture was allowed to stir a total of 3 h. at R.T. before evaporation of solvent under a vacuum. The red solid residue was redissolved in a small quantity of DMF, adsorbed on silica gel and evaporated to dryness under a vacuum. The adsorbed product was then chromatographed on silica gel with chloroform-DMF (9:1). Collection of the major red band gave 27 (30 mg, 88.2%) as a red solid after repeated addition of ethyl

and evaporation under a vacuum to remove traces of DMF:

MP 200-205°C (decomposes); IR 3440, 3320, 3200, 2920, 1690, 1660, 1640; NMR (DMSO-d₆) 6.66 (broad, 2H), 6.39 (broad, 2H) 5.98 (m, 1H), 5.03 (d, 2H), 4.21 (m, 2H), 3.30 (s, H₂O), 2.85 (m, 2H), 2.47 (m, DMSO), 2.24 (s, 4H), 1.94 (s, 3H); MS 374, 270.

Repeated attempts to obtain an elemental analysis gave results inconsistent with the molecular formula despite the overwhelming spectral evidence supporting the designated structure. Hence 27 was purified by semi-preparative scale HPLC as follows: A solution of 27 (15 mg) in DMF-methylene chloride (3:1, 5 mL) was chromatographed (approx. 0.5 mL per run) by eluting first with methylene chloride for 3 min. and then introducing a 0-5% linear gradient of methanol over 20 min. Elution rate was 5 mL/min. Some non-polar material eluted first, followed by DMF. Next to elute were the major pink fraction and two minor components. Center cuts of the major component were collected for each run and combined. A portion (1.0 mL) was then injected and eluted as above to give a single sharp peak on the recorder chart, indicating pure 27. Evaporation of solvent under a vacuum gave 27 as a red solid. The solid was ground to a fine powder and dried under a vacuum at R.T. for 48 h. before submission for elemental analysis. The results were several percent high for C and H and low for N, indicating trapped solvent.

9-Chloromethyl-2,3-dihydro-1-hydroxy-7-methoxy-6-
methyl-1H-pyrrolo[1,2-a]indole-5,8-
dione Methylcarbamate (36)

A solution of 17 (36 mg), methylene chloride (3.5 mL) and acetic anhydride (2.5 mL) was cooled to 0°C and bubbled with anhydrous hydrogen chloride for 5 min. (saturation achieved). The mixture was stirred 10 min. more, then flushed with nitrogen for 10 min. before evaporation at reduced pressure. The residual solid was chromatographed immediately on silica gel, eluting with methylene chloride-acetone (9:1). Collection of a major yellow band gave 34 (24.2 mg, 82.5%) as a peach-colored solid upon evaporation from methylene chloride-hexane: MP 136-138°C (decomposes); IR 3510, 1637; NMR (CDCl₃) 5.34 (d of d, 1H), 4.91 (s, 2H), 4.57 (broad, 1H), 4.31 (t, 2H), 4.00 (s, 3H), 2.65 (m, 2H), 1.97 (s, 3H); MS 295, 297 (3:1), 260. The amount of water present on the silica gel was critical in obtaining the product. Silica gel dried 12 h. at 130°C gave a faster eluting product, theorized to be 33, which decomposed as it left the column. Silica gel direct from the container gave 34 and traces of 35. Silica gel that had been moistened by suspension in methylene chloride and air drying gave 35: MP 164-165°C; IR 3220, 1640; NMR 5.27 (m, 1H), 4.86 (d, 2H), 4.27 (m, 2H), 4.05 (s, 3H), 2.65 (m, 2H), 1.97 (s, 3H); MS 277, 259.

Compound 34 (24 mg, 0.09 mmol) was dissolved in methylene chloride (4 mL) and treated with anhydrous potassium carbonate (750 mg) followed by methyl isocyanate (0.3 mL, 4.9 mmol). The reaction mixture was stirred under argon in a stoppered flask 14 h. at R.T., then the potassium carbonate was filtered and rinsed well with methylene chloride. The combined washings and filtrate were evaporated at reduced pressure and the residue was chromatographed on oven-dried silica gel with methylene chloride-acetone (95:5) to give 36 (21 mg, 72.7%) as a yellow solid: MP 174°C (decomposes); IR 3300, 1690, 1637; NMR (CDCl₃) 6.15 (d of d, 1H), 4.86 (s, 2H), 4.70 (broad, 1H), 4.33 (d of d, 2H), 4.07 (s, 3H), 2.82 (m, 5H), 1.98 (s, 3H); MS 352, 354 (3:1), 259; Anal. calcd. for C₁₆H₁₇N₂O₅Cl: C 54.5, H 4.8, N 7.9; found: C 54.1, H 4.5, N 7.9.

2,3-Dihydro-1-hydroxy-9-hydroxymethyl-7-methoxy-6-methyl-8-nitro-1H-pyrrolo[1,2-a]indole Diacetate (29)

A suspension of 12 (100 mg, 0.30 mmol) in ethanol (25 mL) was treated with sodium borohydride (85 mg, 2.24 mmol) and stirred 1.5 h. at R.T. The reaction was then treated with ice chips and water, stirring vigorously for 10 min. to hydrolyse excess anhydride. The yellow organic fraction was separated, extracted with saturated aqueous cupric sulfate (4 X 50 mL) and water (1 X 100 mL), dried over magnesium sulfate and concentrated at reduced pressure.

The residue was chromatographed on silica gel, eluting first with methylene chloride to collect a minor yellow band then changing the solvent to methylene chloride-acetone (95:5) to collect the major yellow band. Evaporation of solvent from this latter band gave 29 (69 mg, 60.9%) as a yellow, crystalline solid upon evaporation from methylene chloride-hexane: MP 146-147°C; IR 2960, 2915, 2850, 1730, 1520, 1360; NMR (CDCl₃) 7.20 (s, 1H), 6.25 (d of d, 1H), 5.09 (s, 2H), 4.13 (m, 2H), 3.85 (s, 3H), 2.78 (m, 2H), 2.40 (s, 3H), 2.06 (s, 3H), 1.98 (s, 3H); MS 376, 317; Anal. calcd. for C₁₇H₂₀N₂O₇: C 57.4, H 5.3, N 7.4; found: C 57.6, H 5.3, N 7.5.

2,3-Dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1H-pyrrolo[1,2-a]indole-5,8-dione-1-oxime Carbamate (7-Methoxymitosene-1-oxime, 32)

A solution of 19 (50 mg) in methylene chloride (30 mL) was treated with activated manganese dioxide⁷⁴ (500 mg) and stirred 4 h. at R.T. Excess manganese dioxide was collected by filtration and washed well with methanol-methylene chloride (1:1) until no yellow color could be seen in the washings. The combined washings and filtrate were concentrated and the residue was adsorbed on silica gel from methylene chloride solution before drying again at reduced pressure. Chromatography on silica gel with methylene chloride-acetone (3:1) gave 31 (27 mg, 54.3%) as a yellow solid: IR 3480, 3332, 3265, 1720, 1655, 1640.

A solution of 31 (25 mg, 0.08 mmol), pyridine (5 mL), methanol (5 mL) and methylene chloride (12 mL) was treated with hydroxylamine hydrochloride (100 mg, 1.44 mmol) and stirred 4 h. at R.T. During that time the color changed from yellow to bright red. The reaction mixture was diluted with 20 mL methylene chloride, extracted with water (2 X 50 mL), dried over magnesium sulfate and concentrated. The resulting solution in pyridine was diluted with toluene and evaporated under a vacuum to give a maroon solid. Purification was achieved by chromatography on silica gel, after prior adsorption of the product on a small quantity of the gel, by eluting with methylene chloride-acetone-methanol (8:1:1) to give 32 (17.4 mg, 66.5% from 31) as a maroon solid: MP 200°C (slowly decomposes); IR 3600-3520, 1690, 1655, 1640; NMR (DMSO-d₆ + CDCl₃) 6.17 (broad, 2H), 5.11 (s, 2H), 4.34 (m, 2H), 3.94 (s, 3H), 3.25 (s, H₂O), 2.45 (m, DMSO), 1.87 (s, 3H); MS 333, 272.

Compound 32 was troublesome for obtaining a satisfactory elemental analysis so it was purified by semi-preparative scale HPLC as follows: A solution of 32 (5 mg) in methylene chloride-acetone (1:1, 6 mL) was chromatographed (1.0 mL per run) by eluting first with methylene chloride for 6 min. and then introducing a 0-5% linear gradient of methanol over 20 min. The elution rate was 5 mL/min. The acetone eluted first followed by a minor component. Next the major, red-colored component (32) eluted, followed

closely by two minor components. Collection of center cuts from the major peak on each run gave 32 as a red-brown solid upon evaporation of solvent. A small sample was prepared and chromatographed as above to give a single sharp peak on the recorder chart, indicating pure 32. After vacuum drying at R.T. for 24 h. the purified 32 was submitted for elemental analysis: Anal. calcd. for $C_{15}H_{15}N_3O_6$: C 54.1, H 4.5, N 12.6; found: C 54.0, H 4.5, N 12.1.

Comparative Acid Hydrolysis of Mitosenes

In three concurrent experiments, compounds 17, 20 and 25 (2.0 mg) were each treated with methanol (2.0 mL), stirred 15-20 min. to dissolve, filtered to remove any undissolved material and then treated with 0.3 N hydrochloric acid (0.5 mL). TLC (chloroform-acetone, 7:3) was examined at intervals parallel to compound 19 and compared with a TLC chromatogram taken before acid addition.

After 10 min. a trace of 19 was clearly visible in the reaction of 25. This grew to be the major spot after 45 min. and then other spots began to appear, the largest of which remained at the origin. At 60 min. no 25 was visible on the TLC chromatogram.

The reaction mixture of 17 remained unchanged until after about 60 min when polar impurities began to appear at the origin. After 120 min., a spot corresponding

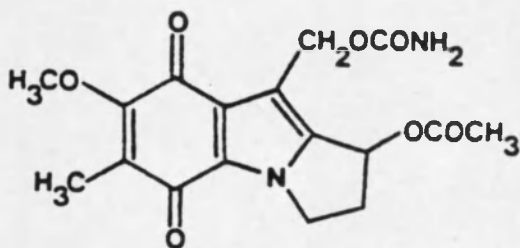
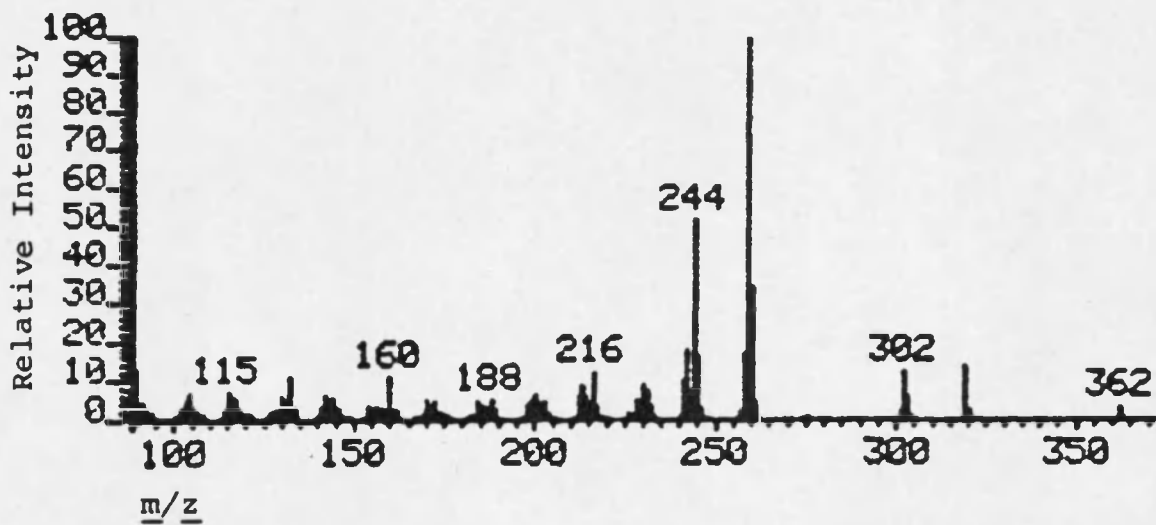
to 17 was still visible but there was at no time any evidence of 19.

The behavior of compound 20 closely resembled that of 17 except that a trace of a compound with an R_f value close to 19 began to appear after 60 min. This was still a very minor component at 120 min.

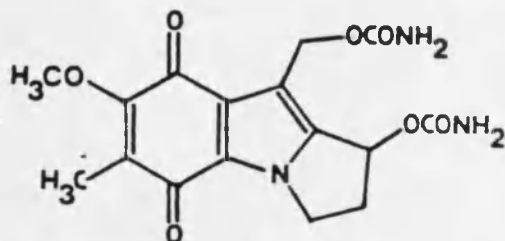
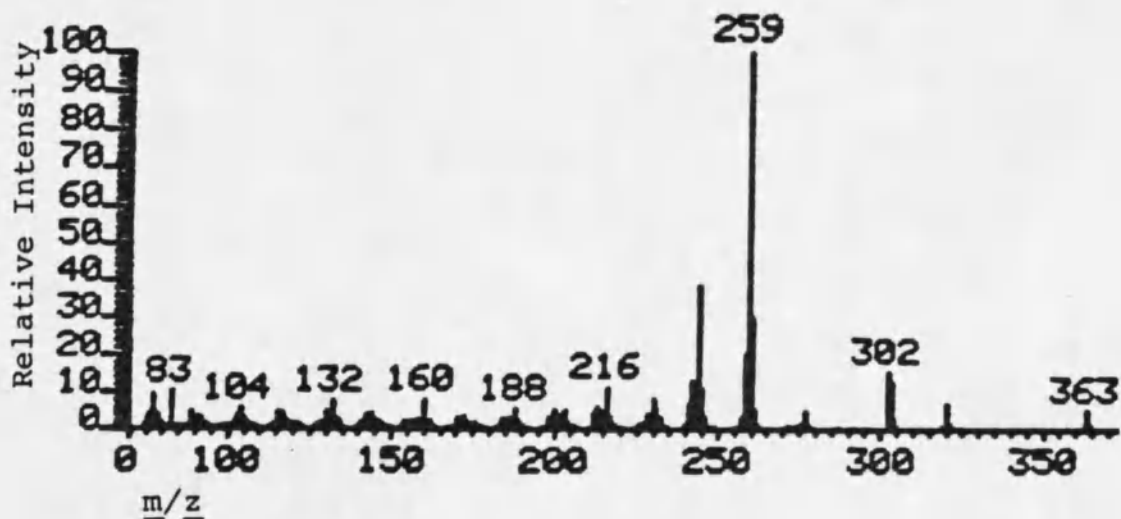
Acid Hydrolysis of 1-Hydroxy-
7-methoxymitosene Nicotinate (25)

A solution of 25 (3.0 mg) in 1.0 N hydrochloric acid (10 mL) was suspended over methylene chloride (10 mL). At 3, 5, 7, 9, 12, and 15 min. after the original dissolution of the starting material the mixture was shaken vigorously and the layers allowed to separate. The orange color rapidly moved from the aqueous to the organic layer and at 15 min. the former was nearly colorless. TLC (methylene chloride-acetone, 6:1) of the organic layer showed only 19 when compared to reference samples of 25 and 19. The experiment was repeated on a larger scale with 3 N hydrochloric acid and an NMR spectrum of the evaporated organic layer was identical to 19.

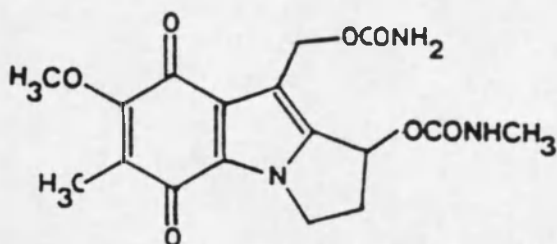
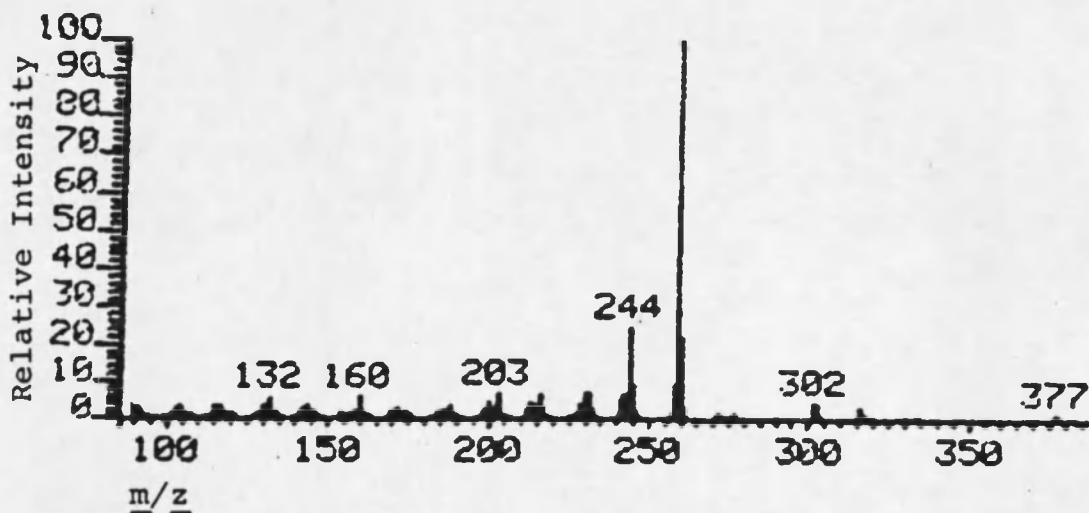
APPENDIX A:
MASS SPECTRAL DATA



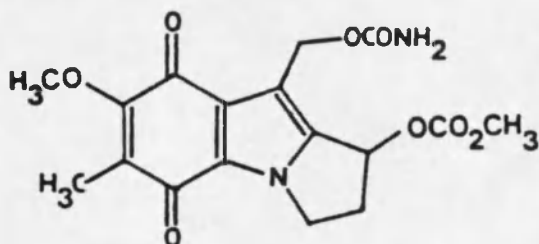
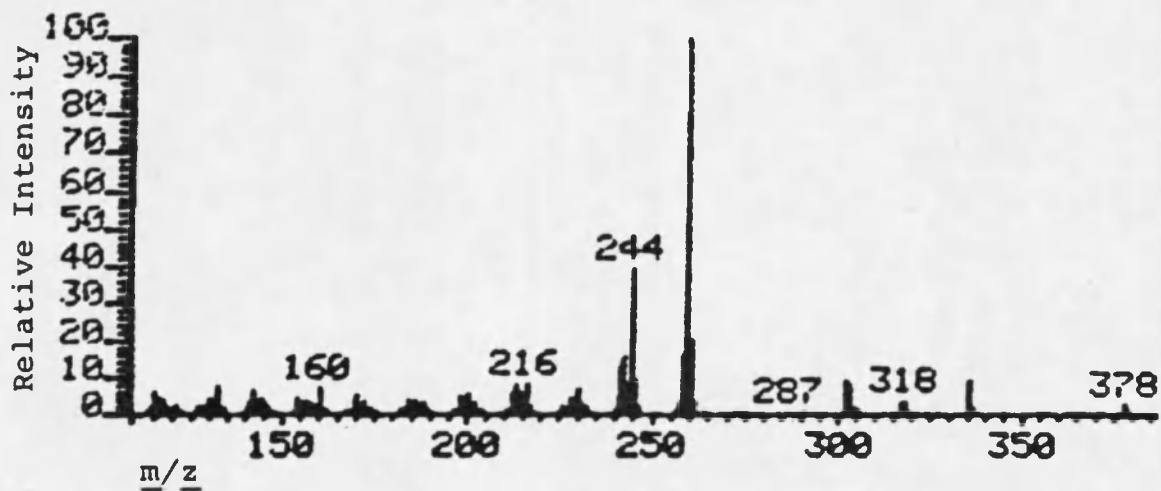
Compound	m/z	Relative Intensity	Identity
<u>17</u>	362	3.10	M^+
	319	13.85	M^+ - $HNCO$
	320	2.47	M^+ - CH_2CO
	303	5.97	M^+ - $CH_3CO_2^{\cdot}$
	302	12.47	M^+ - CH_3CO_2H , M^+ - $H_2NCO_2^{\cdot}$
	301	2.29	M^+ - H_2NCO_2H
	259	100.00	B^+
	244	51.57	B^+ - CH_3^{\cdot}



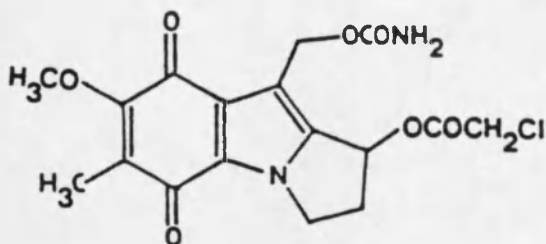
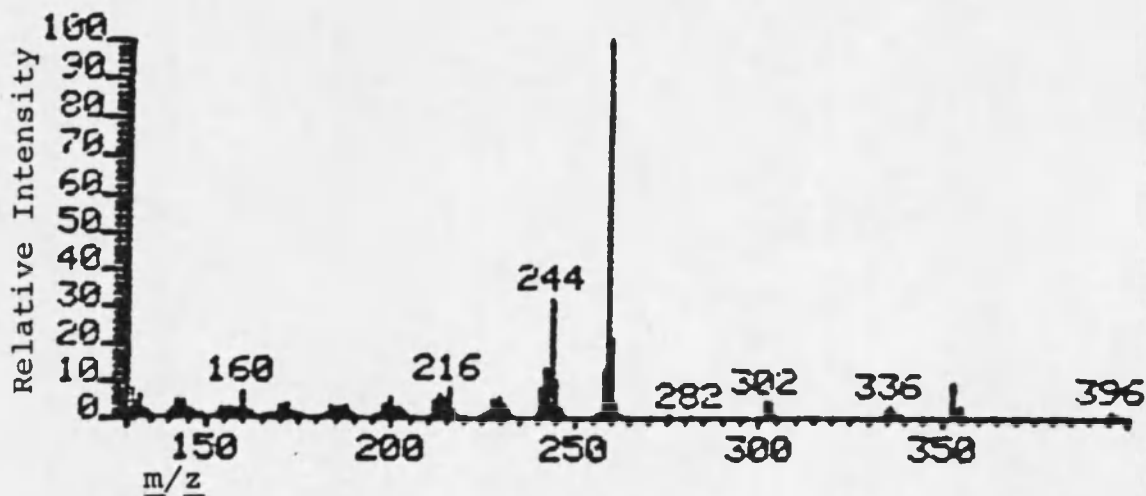
Compound	m/z	Relative Intensity	Identity
<u>20</u>	363	5.08	M^+
	320	6.76	M^+ - $HNCO$
	303	15.36	M^+ - $H_2NCO_2\cdot$
	302	12.40	M^+ - H_2NCO_2H
	277	4.58	M^+ - $2(HNCO)$
	259	100.00	B^+
	244	38.60	B^+ - $CH_3\cdot$



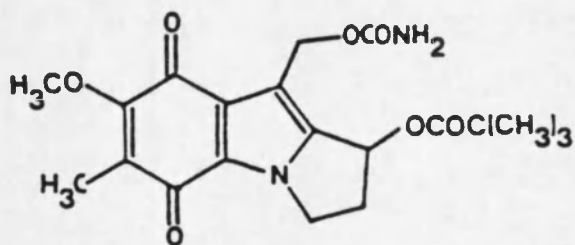
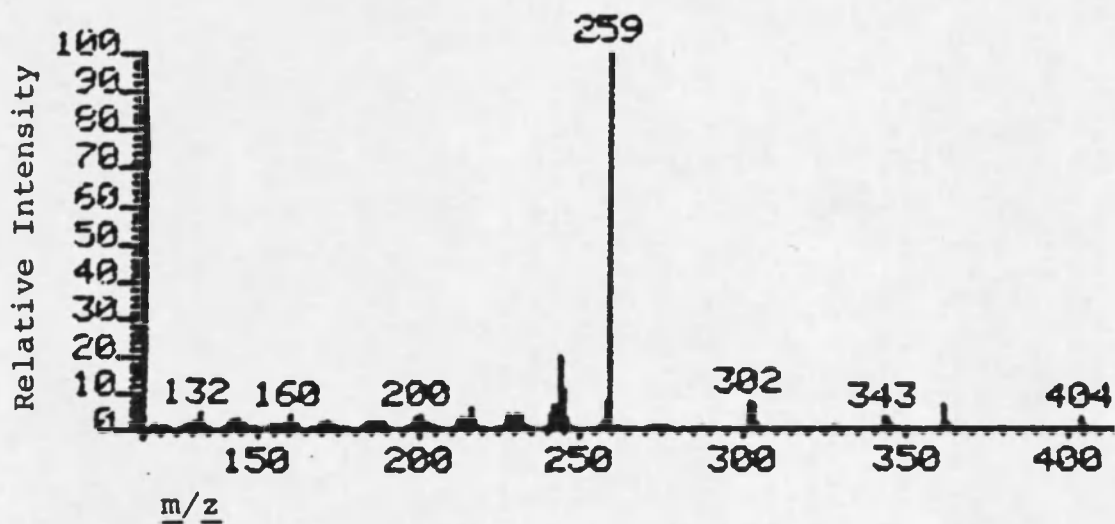
Compound	m/z	Relative Intensity	Identity
<u>21</u>	377	1.31	M^+
	334	0.54	M^+ - HNCO
	320	0.29	M^+ - CH_3NCO
	317	1.48	M^+ - $\text{H}_2\text{NCO}_2 \cdot$
	316	3.05	M^+ - $\text{H}_2\text{NCO}_2\text{H}$
	303	3.93	M^+ - $\text{CH}_3\text{NHCO}_2 \cdot$
	302	4.04	M^+ - $\text{CH}_3\text{NHCO}_2\text{H}$
	277	1.23	M^+ - $(\text{CH}_3\text{NCO} + \text{HNCO})$
	259	100.00	B^+
	244	24.44	B^+ - $\text{CH}_3 \cdot$



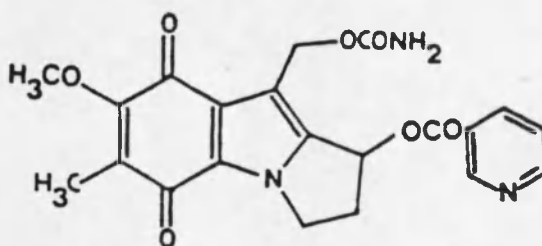
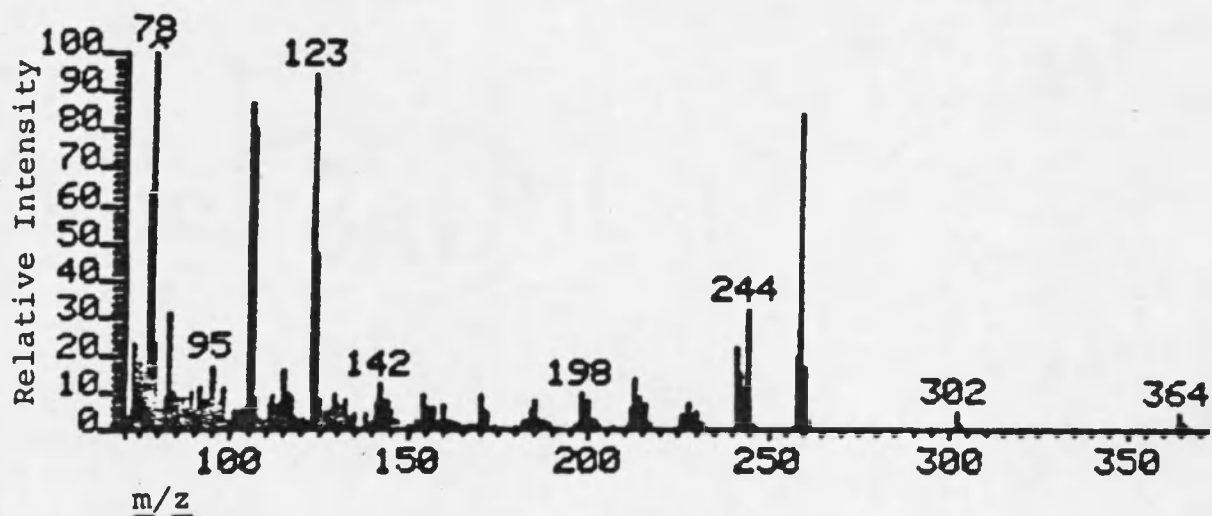
Compound	m/z	Relative Intensity	Identity
<u>22</u>	378	2.45	M^+
	335	9.01	M^+ - $HNCO$
	318	3.48	M^+ - $H_2NCO_2^{\cdot}$
	317	2.80	M^+ - H_2NCO_2H
	303	4.08	M^+ - $CH_3OCO_2^{\cdot}$
	302	3.99	M^+ - CH_3OCO_2H
	259	100.00	B^+
	244	32.15	B^+ - CH_3^{\cdot}



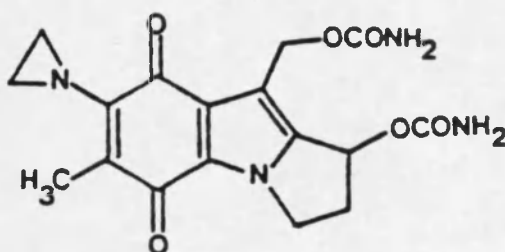
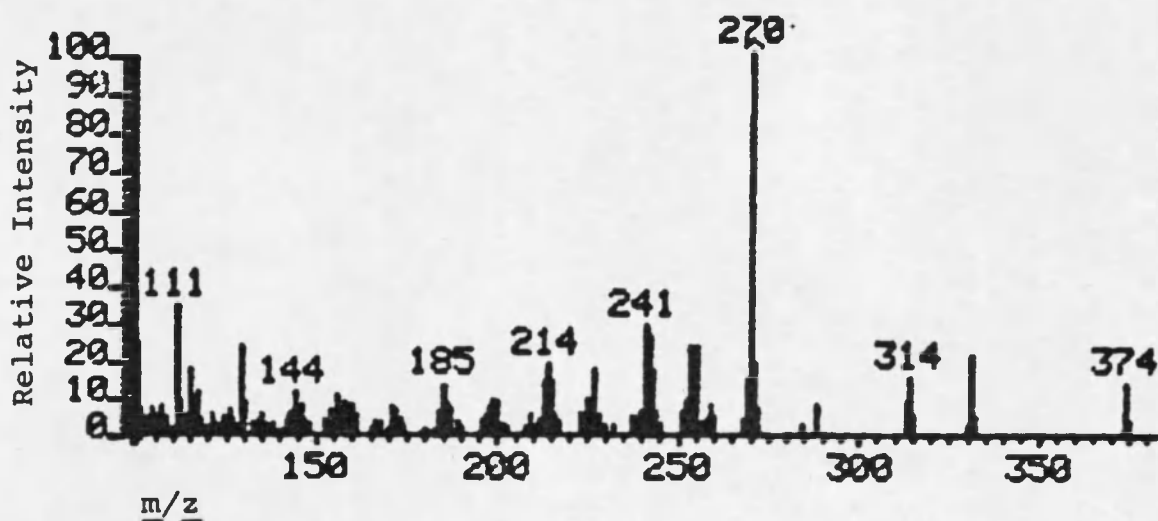
Compound	m/z	Relative Intensity	Identity
<u>23</u>	398	0.45	$\text{M}^+ + 2$
	396	1.41	M^+
	355	2.96	$(\text{M}^+ + 2) - \text{HNCO}$
	353	9.10	$\text{M}^+ - \text{HNCO}$
	338	0.81	$(\text{M}^+ + 2) - \text{H}_2\text{NCO}_2\cdot$
	337	1.34	$(\text{M}^+ + 2) - \text{H}_2\text{NCO}_2\text{H}$
	336	2.77	$\text{M}^+ - \text{H}_2\text{NCO}_2\cdot$
	335	1.64	$\text{M}^+ - \text{H}_2\text{NCO}_2\text{H}$
	303	3.99	$\text{M}^+ - \text{ClCH}_2\text{CO}_2\cdot$
	302	4.08	$\text{M}^+ - \text{ClCH}_2\text{CO}_2\text{H}$
	259	100.00	B^+
	244	32.15	$\text{B}^+ - \text{CH}_3\cdot$



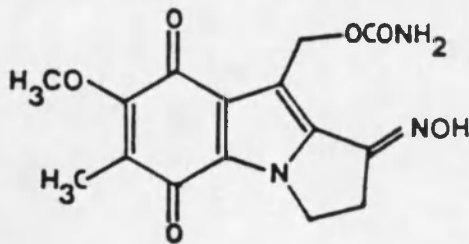
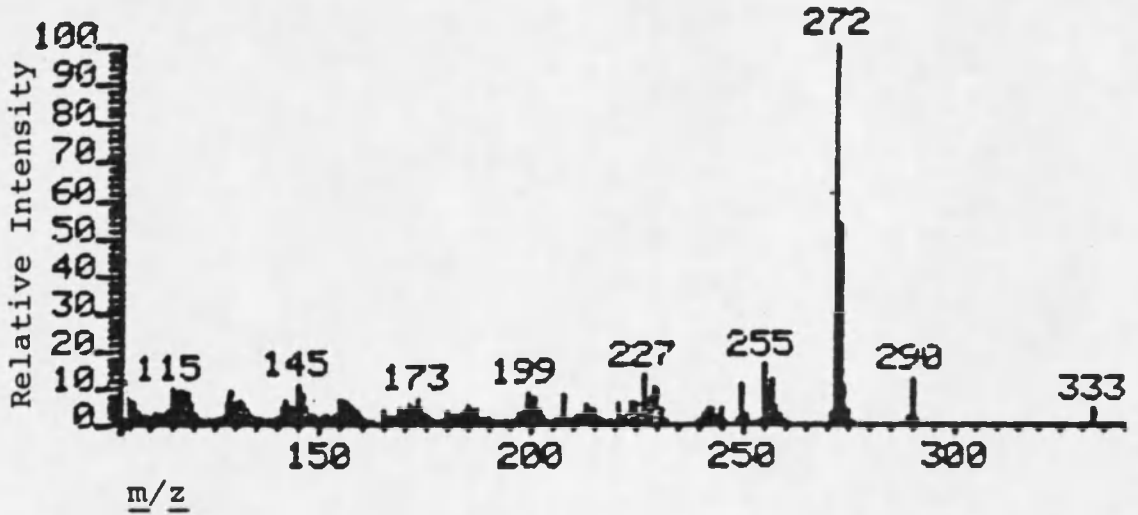
Compound	m/z	Relative Intensity	Identity
<u>24</u>	404	2.82	M^+
	361	6.11	M^+ - $HNCO$
	344	2.45	M^+ - $H_2NCO_2 \cdot$
	343	2.98	M^+ - H_2NCO_2H
	303	6.63	M^+ - $(CH_3)_3CCO_2 \cdot$
	302	7.07	M^+ - $(CH_3)_3CCO_2H$
	259	100.00	B^+
	244	19.56	B^+ - $CH_3 \cdot$



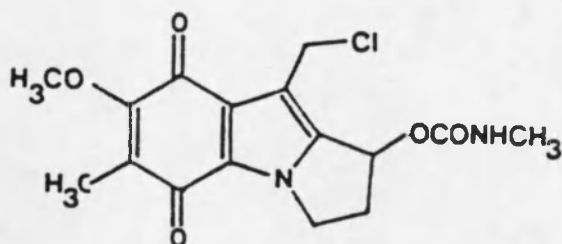
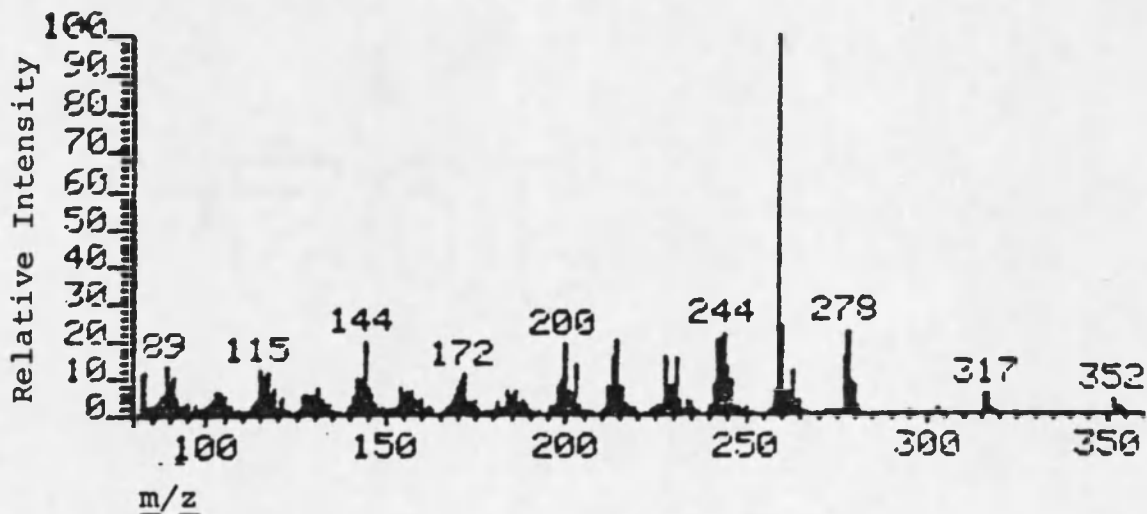
Compound	<u>m/z</u>	Relative Intensity	Identity
<u>25</u>	425	0.00	M ⁺
	365	1.62	M ⁺ - H ₂ NCO ₂ [·]
	364	4.10	M ⁺ - H ₂ NCO ₂ H
	303	0.71	M ⁺ - (C ₅ H ₄ N)CO ₂ [·]
	302	4.66	M ⁺ - (C ₅ H ₄ N)CO ₂ H
	259	84.22	M ⁺ - (HNCO + (C ₅ H ₄ N)CO ₂ H)
	244	32.16	259 ⁺ - CH ₃ [·]
	123	94.09	(C ₅ H ₄ N)CO ₂ H ⁺
	78	100.00	C ₅ H ₄ N ⁺



Compound	m/z	Relative Intensity	Identity
<u>27</u>	374	13.21	M^+
	331	20.47	M^+ - $HNCO$
	314	13.73	M^+ - $H_2NCO \cdot$
	313	8.54	M^+ - H_2NCO_2H
	288	7.33	M^+ - $2(HNCO)$
	270	100.00	B^+
	242	25.20	B^+ - C_2H_4
	241	27.59	B^+ - $C_2H_5 \cdot$



Compound	<u>m/z</u>	Relative Intensity	Identity
<u>32</u>	333	4.26	M ⁺
	290	11.01	M ⁺ - HNC=O
	273	50.89	M ⁺ - H ₂ NCO ₂ ·
	272	100.00	M ⁺ - H ₂ NCO ₂ H = B ⁺
	257	12.30	B ⁺ - CH ₃ ·
	255	17.35	B ⁺ - OH·



Compound	m/z	Relative Intensity	Identity
<u>36</u>	354	1.65	$M^+ + 2$
	352	3.87	M^+
	317	5.29	$M^+ - Cl \cdot$
	316	4.97	$M^+ - HCl$
	280	7.72	$(M^+ + 2) - RCO_2 \cdot$
	279	8.84	$(M^+ + 2) - RCO_2H$
	278	22.05	$M^+ - RCO_2 \cdot$
	277	14.71	$M^+ - RCO_2H$
	259	100.00	B^+
	244	21.53	$B^+ - CH_3 \cdot$
	243	20.03	$278^+ - Cl \cdot$
	242	20.03	$277^+ - Cl \cdot$

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