Mass Spectra of Synthetic Mitosenes

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The mass spectral fragmentation of a series of 10 mitosene analogs is described. Major pathways arise through decomposition of the substituents in the 1 and 10 positions and are unaffected by the substituent in the 7 position. The base peak in the spectrum, in most cases, is represented by the basic mitosene ring. The patterns are general for this series of compounds and may have application in elucidating the structure of the DNA alkylation sites involved in reactions with mitosenes or natural mitomycins.

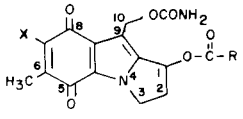
J. Heterocyclic Chem., **19**, 161 (1982).

During the course of our recent studies (4) concerning the total synthesis and antineoplastic screening of mitosene analogs of the mitomycin antibiotics (5), we made extensive use of mass spectral data for strucutre verification. As a result, the characteristic fragmentation pattern of the mitosenes shown in Table 1 was elucidated utilizing mass shift observations to provide insight for the relatively simple degradation processes observed. High resolution studies were employed for selected compounds to verify fragment compositions.

The synthetic mitosenes prepared differ from the naturally occurring antibiotics **(11-14)** (Table 2) by exclusion of the elements of methanol from the 9 and 9a positions and by the substitution of novel 1-substituents for the 1,2-aziridine ring. The fragmentation patterns of mitomycins and semisynthetic derivatives have been reported by VanLear (6). The presence of the aziridine functionality and 9a-substituents dominate the fragmentation in these cases and as a result the mass spectra for our mitosenes differ substantially from naturally derived mitomycins.

Table I

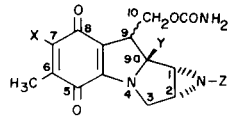
Structures of Synthetic Mitosenes
Whose Mass Spectra Have Been Studied (9)



		R	X
1,	1-Acetoxy-7-methoxymitosene	CH_3	OCH_3
2,	1-Hydroxy-7-methoxymitosene carbamate	NH_2	OCH_3
3,	1-Hydroxy-7-methoxymitosene methylcarbamate	$NHCH_3$	OCH_3
4,	1-Hydroxy-7-methoxymitosene methylcarbonate	OCH_3	OCH_3
5,	1-Hydroxy-7-methoxymitosene chloroacetate	CH_2Cl	OCH_3
6,	1-Hydroxy-7-methoxymitosene trimethylacetate	$C(CH_3)_3$	OCH_3
7,	1-Hydroxy-7-methoxymitosene nicotinate		OCH_3
8,	7-(1-Aziridinyl)-1-hydroxymitosene acetate	CH_3	
9,	7-(1-Aziridinyl)-1-hydroxymitosene carbamate	NH_2	
10,	9-Chloromethyl-2,3-dihydro-1-hydroxy-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]-indole-5,8-dione methylcarbamate	$NHCH_3$	10 = CH_2Cl

Table 2

Structures of Natural Occurring Mitomycins

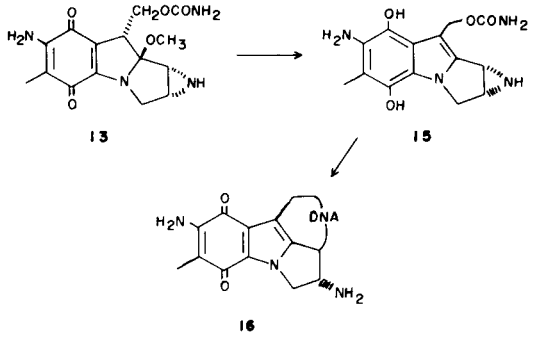


Compound		X	Y	Z	9-Substituent
11	Mitomycin A	CH_3O-	-OCH_3	-H	α
12	Mitomycin B	CH_3O-	-OH	-CH_3	β
13	Mitomycin C	H_2N-	-OCH_3	-H	α
14	Porfiromycin	H_2N-	-OCH_3	-CH_3	α

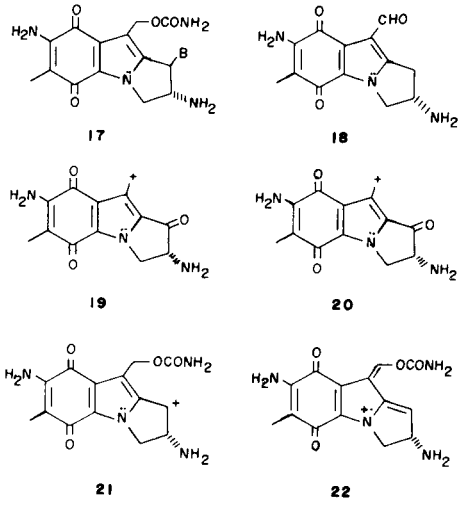
In light of the current interest in the mechanism and locus of action for the native mitomycins as antineoplastic agents, the mass spectral fragmentation patterns of our synthetic mitosenes are of particular value since mitosene moieties have been proposed as units of alkylation *in vivo*. One of the currently popular theories for the cytotoxic action of mitomycins is that of bioreductive alkylation. The body of evidence in support of this mechanism has been reviewed extensively (7,8). In brief, the bioreductive alkylation sequence for mitomycin C (**13**) is shown in Scheme 1. Upon enzymatic reduction of the quinone, mitomycin C is proposed to eliminate the elements of methanol to give the active alkylating agent **15**. Nucleophilic groups on DNA then effect the opening of the aziridine ring and the displacement of carbamate to give cross-alkylated DNA. Spontaneous reoxidation to the indoloquinone **16** results in the net effect of covalent binding of mitosene units to DNA. It has been proposed that bifunctional aklylation is most toxic when complimentary strands of DNA are linked together and that such DNA cross linking is a requirement for antineoplastic action even though monofunctional alkylations have also been demonstrated.

Scheme 1

Bioreductive Alkylation of Mitomycin C



Despite considerable evidence for the alkylating activity of mitomycins, the exact sites of attachment to DNA have not been established. For both *in vitro* and *in vivo* DNA alkylation experiments, the sensitivity of mass spectral analysis will surely be required for structure elucidation of alkylated species. In this respect the fragmentation pattern of synthetic, substituted mitosenes should be more valuable as a model for the fragmentation of mitosene units on alkylated DNA than the fragmentation patterns of either the natural mitomycins or semi-synthetic aziridinomitosenes. For example, a reasonable structure for a monoalkylated DNA base residue is shown in structure **17** below. The identity of the base would be indicated by the $M^{+\cdot}$, $M^{+\cdot}$-HNCO, $M^{+\cdot}$-$H_2NCO_2\cdot$ and $M^{+\cdot}$-H_2NCO_2H ions which, based on the fragmentation of the mitosene model compounds, should be easily observed. If *O*-alkylation occurred in the DNA base moiety, an ion at m/z 259 arising from a combination of **18** and **19** could be observed whereas *N*-alkylation could give **20**. The appearance of ions at m/z 302 and 303 (**21** and **22**, respectively) would confirm substitution in the 1-position of the mitosene nucleus.



The low resolution mass spectra of compounds **1**, **8** and **10** are shown in Figure 1 and a listing of structurally significant ions in the spectra of compounds **1**-**10** are included in Table 3. In all but one of the compounds analyzed, a molecular ion was detected using electron impact ionization. The molecular ion is characteristically a small

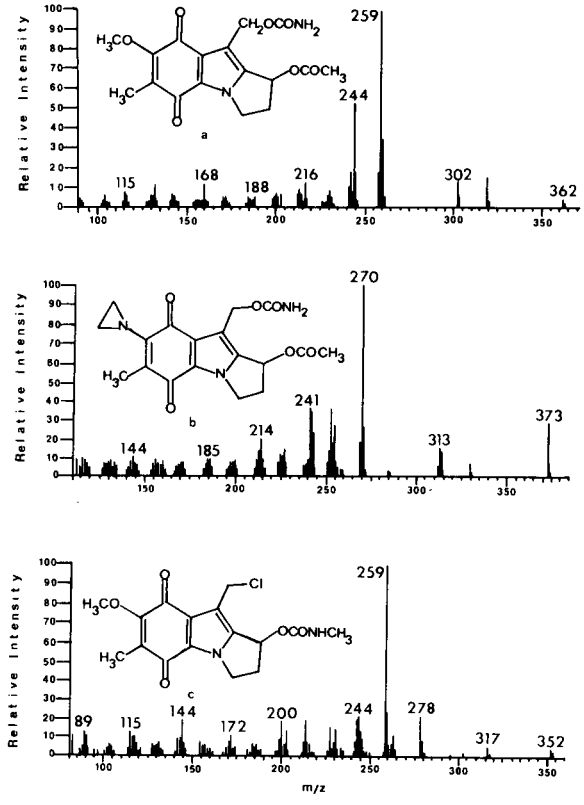


Figure 1. Low resolution mass spectra of compounds **1** (a), **8** (b), and **10** (c) obtained at 70 eV ionizing voltage.

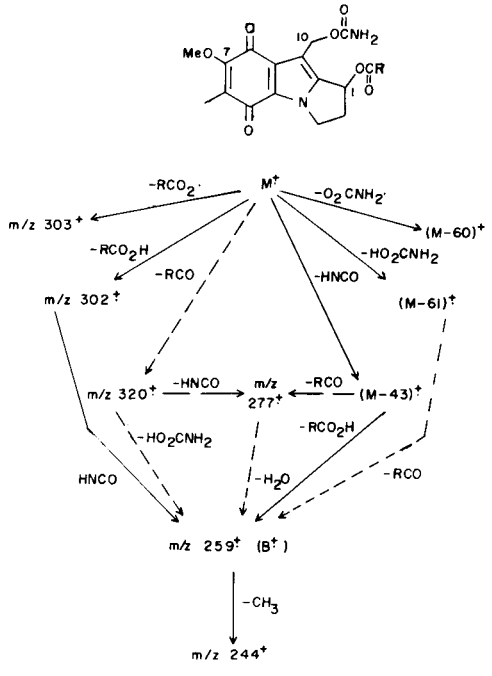
peak with a relative intensity less than ten percent. The usual pattern of breakdown consists of a few predictable low intensity ions until one reaches the base peak which is dependent on the 7-substituent but independent of the 1-and 10-substituents. Below the base peak there are usually one or two identifiable ions of moderate intensity and many low intensity ions that are difficult to identify. General fragmentation schemes are presented in Schemes 2 and 3 for compounds containing a 7-methoxy or 7-aziridinyl group, respectively, and analogous pathways are proposed for the other members in the series of substituted mitosenes investigated. High resolution data confirms the elemental composition of the major ions in the spectra of **1** and **8** and, based on these data, the structures of the major fragment ions may be represented as shown in Scheme 4 for compound **1**.

Concentrating on 7-methoxymitosene derivatives (Scheme 2) one can see that decomposition of the molecular ion can take place by loss of either the 10- or 1-substituent. The 10-carbamate is lost either by elimination of isocyanic acid, carbamic acid or carbamate radical. Simi-

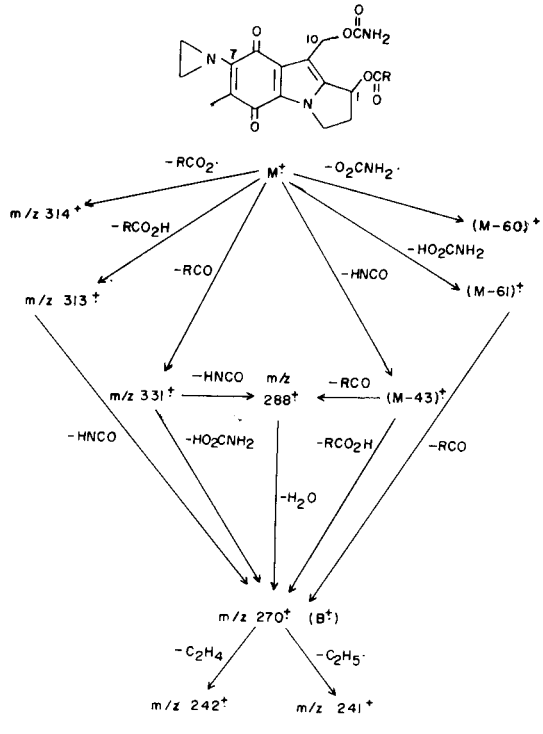
Table 3
Major Ions in the Mass Spectra of Compounds **1-10**

Assignment	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
$M^{+\cdot}$	362 (3.1)	363 (5.1)	377 (1.3)	378 (2.4)	396 (1.4)	404 (2.8)		373 (29.7)	374 (13.2)	352 (3.9)
M-R=C=O	320 (2.5)	320 (6.8)	320 (0.3)					331 (1.5)		
M-HNCO	319 (13.8)	320 (6.8)	344 (0.5)	335 (9.0)	353 (9.1)	361 (6.1)		330 (6.5)	331 (20.5)	
$M-H_2NCO_2\cdot$	302 (12.5)	303 (15.4)	317 (1.5)	318 (3.5)	336 (2.8)	344 (2.4)	365 (1.6)	313 (14.8)	314 (13.7)	
$M-H_2NCO_2H$	301 (2.3)	302 (12.4)	316 (3.1)	317 (2.8)	335 (1.6)	343 (3.0)	364 (4.1)	312 (5.5)	313 (8.5)	
$M-RCO_2\cdot$	303 (6.0)	303 (15.4)	303 (3.9)	303 (4.1)	303 (4.0)	303 (6.6)	303 (0.7)	314 (12.2)	313 (8.5)	278 (22.0)
$M-RCO_2H$	302 (12.5)	302 (12.4)	302 (4.0)	302 (4.0)	302. (4.1)	302 (7.1)	302 (4.7)	313 (14.8)	313 (8.5)	277 (4.7)
M-(R=CO + HNCO)		266 (4.6)	277 (1.2)						288 (7.3)	
$B^{+\cdot}$	259 (100.0)	259 (100.0)	259 (100.0)	259 (100.0)	259 (100.0)	259 (100.0)	259 (84.2)	270 (100.0)	270 (100.0)	259 (100.0)
$B-CH_3\cdot$	244 (51.6)	244 (38.6)	244 (24.4)	244 (32.2)	244 (32.1)	244 (19.6)	244 (32.2)			244 (21.5)
$B-C_2H_4$							242 (35.1)	242 (25.2)		
$B-C_2H_5\cdot$								241 (35.8)	241 (27.6)	
Other							123 (94.1) $[(C_6H_4N)CO_2H]^{+\cdot}$ 78 (100.0) $[C_5H_4N]^+$			317 (5.3) [M-Cl]· 316 (5.0) $[M-HCl]^{+\cdot}$

Scheme 2
Fragmentation Pathways of 7-Methoxy Substituted Mitosene Compounds



Scheme 3
Fragmentation Pathways of 7-(1-Aziridinyl) Substituted Mitosene Compounds



larly 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. This ion may actually arise by two separate mechanisms and may have the two structures proposed in Scheme 4. Isotope labeling experiments using ^{18}O would be required to confirm the presence, or absence, of specific oxygens. In those compounds that can not lose a ketene or an isocyanate from the 1-position, for example **3**, **6** and **7**, the pattern is simplified somewhat as shown by the dashed arrows in Scheme 2. The base peak remains at m/z 259, however. Another charcteristic 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. Occasional-

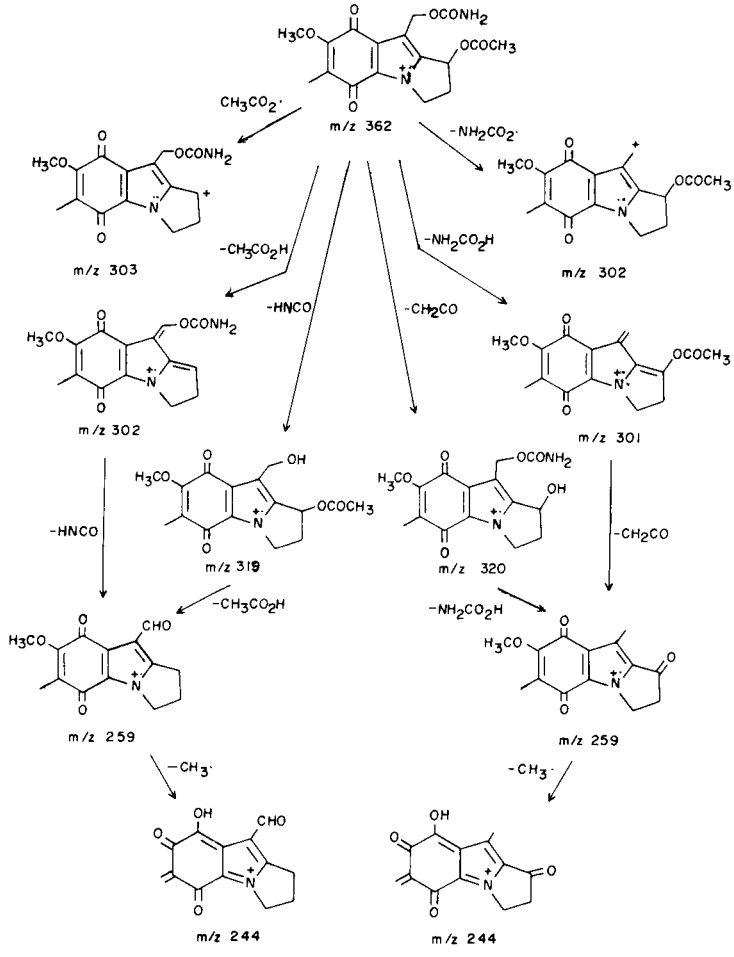
ly one can see the combination of loss of isocyanic acid from the 10-position plus loss of ketene (or isocyanate) from the 1-position to give m/z 277 but this fragment is of low intensity and is not seen consistently.

In the case of 7-(1-aziridinyl)mitosenes (Scheme 4) the fragmentation pattern is the same except for mass shifts due to the weight difference of the 7-substituent and 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 expect that other 7-substituents would also not affect decomposition of the parent ion except to 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 **10** (Figure 1c and Table 3). One simply sees the loss of chlorine radical and hydrogen chloride 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, also leading to a base peak at m/z 259. This is an interesting proposition since it could be extended to cover other 1- and 10-substituents such as those that might arise from alkylation of biomolecules. Monofunctional alkylation of a nucleotide, for example, would give predictable fragment ions based on the mitosene portion of the alkylated product, provided a heteroatom has been alkylated. Bifunctional alkylation would not necessarily give the exact m/z values seen with the mitosene model. Alkylation of two nitrogen atoms may alter the composition of the mitosene-derived base peak even though the fragmentation processes would be expected to be similar. Thus by focusing on mitosene fragments a significant amount of structural information may be obtained

Scheme 4

Proposed Fragment Structures for
1-Acetoxy-7-methoxymitosene (**1**)



concerning the alklylation sites on DNA by using a cominbation of EI and CI mass spectrometery.

EXPERIMENTAL

Low and high resolution mass spectra were obtained using a Varian 311A mass spectrometer. Low resolution spectra were acquired at R = 1000 and high resolution data was obtained at R = 7000 using a Varian-SS200 data system. Scan speeds were 5 sec/dec and 25 sec/dec for low and high resolution, respectively. High resolution data were within ±10 millimass units of the calculated exact mass for the theoretical composition. All spectra were obtained at 70 eV ionizing voltage, a source temperature of 250° and 2 mA filament current. Samples were introduced by a direct insertion probe which was heated from ambient to 240° in 5 seconds.

Acknowledgments.

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REFERENCES AND NOTES

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(9) Correct elemental analysis of **1** has been reported earlier (10). Analysis of **2-8** and **10** are reported in reference 5. Numerous attempts to obtain an acceptable analysis of **9** have been unsuccessful as described in reference 5.

(10) G. Leadbetter, D. L. Fost, N. N. Ekwuribe and W. A. Remers, *J. Org. Chem.*, **39**, 3580 (1974).