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MASS SPECTROMETRIC STUDIES OF ANTIBIOTICS—I
MASS SPECTRA OF MITOMYCIN ANTIBIOTICS:

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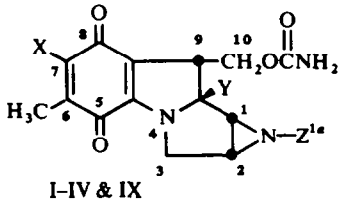
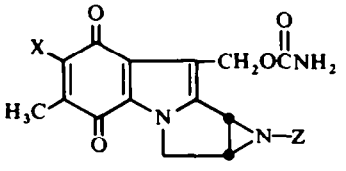
Abstract—The mass spectra of the four known mitomycin antibiotics, mitomycin A, B, C, porfiromycin, and four related compounds are reported. Characteristic fragmentation patterns for this group of antibiotics are discussed. The nature of major fragment ions from the aziridine ring has been established *via* deuterium labeling.

THE mitomycin antibiotics, mitomycin A (I), mitomycin B (II), mitomycin C (III), and porfiromycin (IV),[1–3] are broad spectrum antibiotics which are the first naturally occurring compounds containing an aziridine ring,[4] a pyrrolo (1.2a] indole ring system, an aminobenzoquinone, and pyrrolizine group.[5,6] Although the pharmacological properties of the mitomycins have not been exploited in the United States, mitomycin C is an accepted drug in Japan. We have undertaken a mass spectrometric study of members of this class of antibiotics and of several related analogs in order to determine the general fragmentation behavior of the known compounds. In addition, we wished to determine the usefulness of mass spectrometry for ascertaining the structure of unassigned members of the mitomycin family.[3,7] We felt mass spectrometry would be very useful in this regard since the known mitomycins (I–IV) contain in common the mitosane (IX) ring system with a limited variety of substituents.



I–IV & IX



VII & VIII

		X	Y	Z
I	Mitomycin A	MeO	OMe	H
II	Mitomycin B	MeO	OH	Me
III	Mitomycin C	H_2N	OMe	H
IV	Porfiromycin	H_2N	OMe	Me
V	N-methyl-I	MeO	OMe	Me
VI	N-trideuteromethyl-I	MeO	OMe	CD_3
VII	Anhydro-*apo*-mitomycin B	MeO	—	Me
VIII	Anhydro-*apo*-porfiromycin	H_2N	—	Me
IX	Mitosane	H	H	H

RESULTS AND DISCUSSION

The mass spectra of the four mitomycin antibiotics (I–IV) (Figs 1–4) and related analogs (V–VIII) (Figs 5–8) were obtained. All were volatile and gave spectra in the

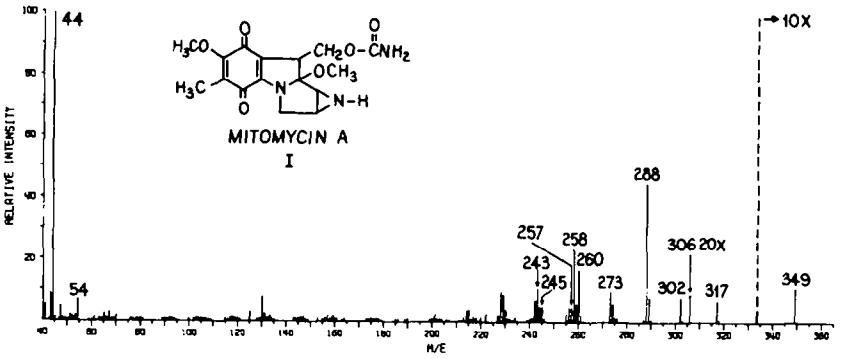


FIG. 1

Mass spectrum of Mitomycin A(I).

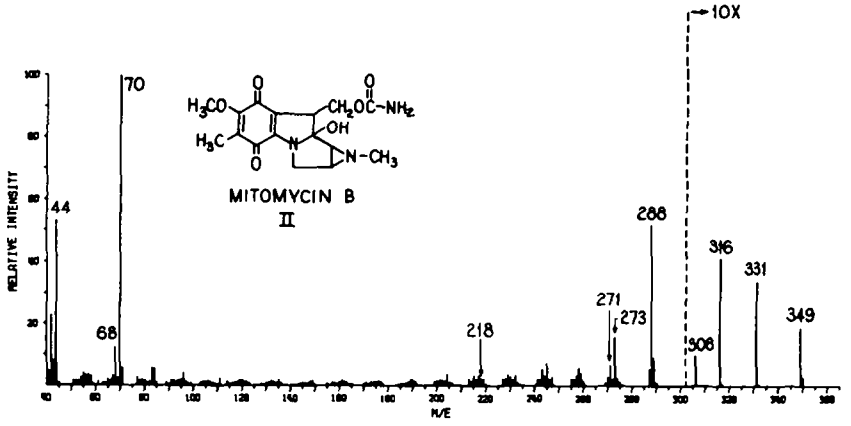


FIG. 2

Mass spectrum of mitomycin B (II).

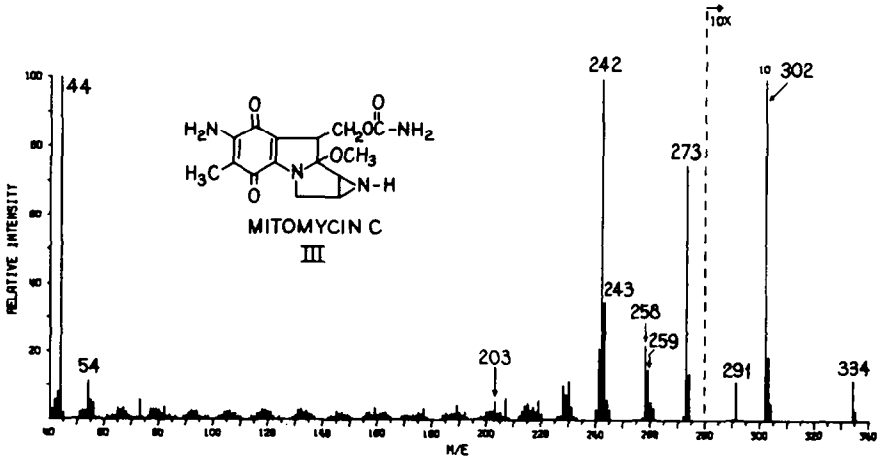


FIG. 3

Mass spectrum of mitomycin C (III).

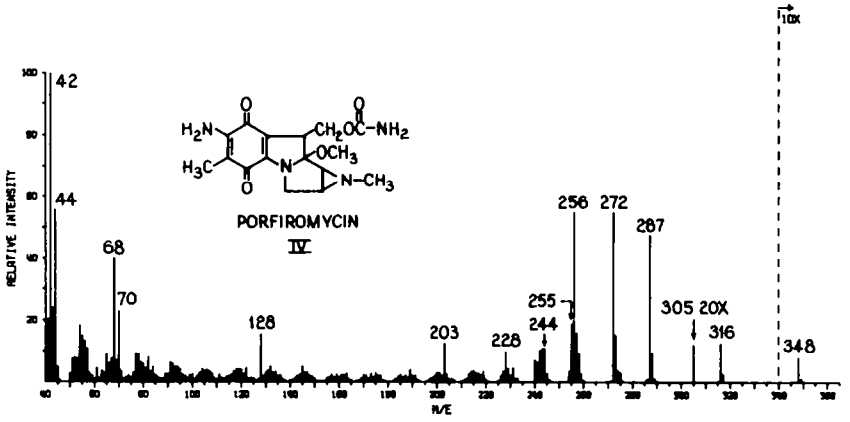


FIG. 4.

Mass spectrum of porfiromycin (IV).

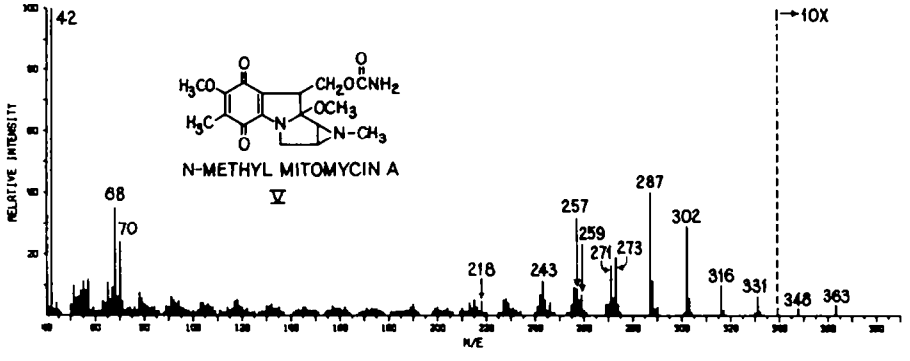


FIG. 5

Mass spectrum of N-methylmitomycin A (V).

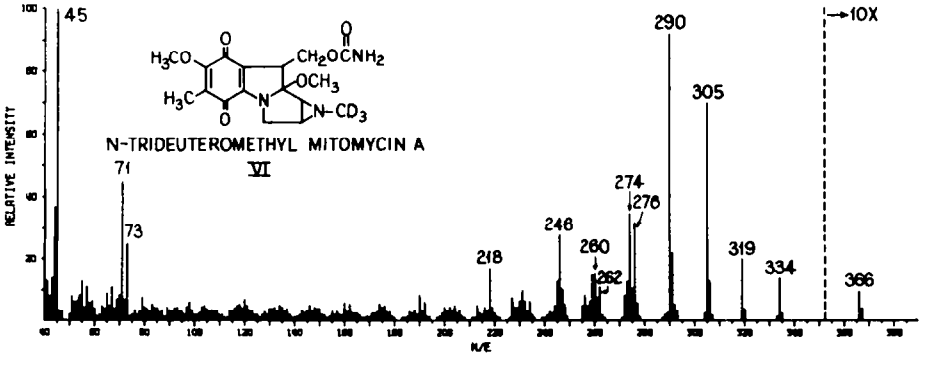


FIG. 6

Mass spectrum of N-trideuteromethylmitomycin A (VI).

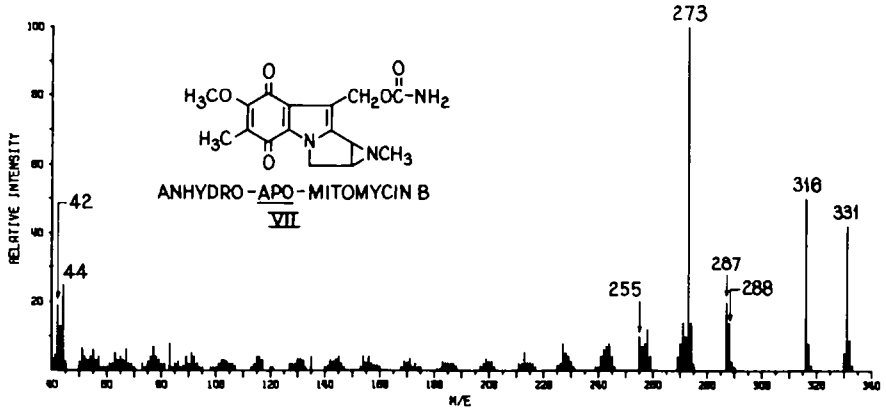


FIG. 7

Mass spectrum of anhydro-*apo*-mitomycin B (VII).

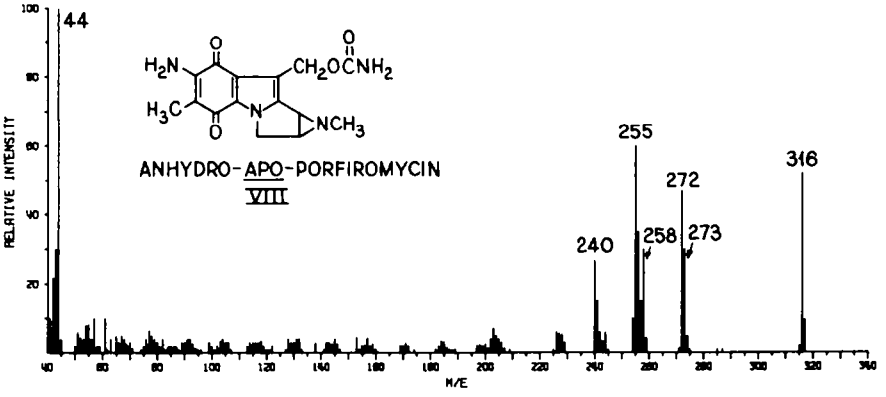


FIG. 8

Mass spectrum of anhydro-*apo*-porfiromycin (VIII).

absence of thermal decomposition. The spectra correlated quite well and since mitomycin A and mitomycin B differ by the placement of a $-CH_2-$ unit and porfiromycin contains one $-CH_2-$ group more than mitomycin C, the mass shift technique[8] was used in some instances to provide insight to particular fragmentation processes.

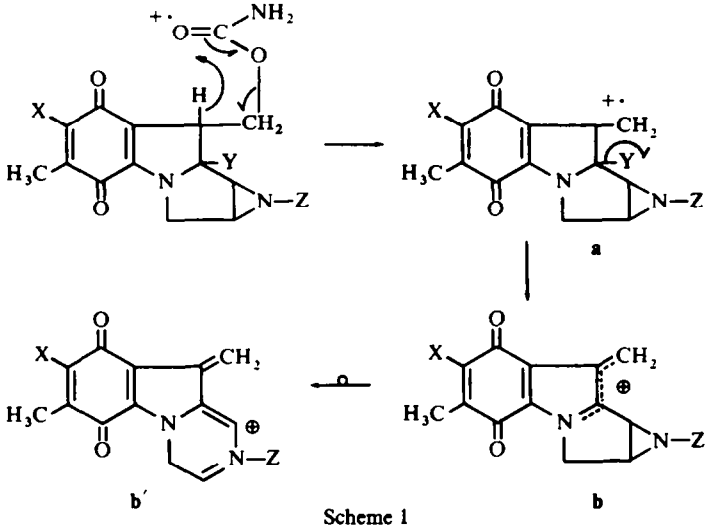
Compounds I–VI having the mitosane (IX) ring system[5] exhibit weak molecular ions. However, strong molecular ions were observed for members of the anhydro-*apo*-class, VII and VIII. The M+2 peaks which have been reported to be quite prominent in other benzoquinone and naphthoquinone systems[9] were not observed in the spectra of any of the samples in our study.

The naturally occurring compounds (I–IV) exhibit similar behavior in the high mass region (Table 1) and mitomycin B (II) is discussed as a model. The first prominent fragment peak below the molecular ion (*m/e* 349) is observed at *m/e* 331 and corresponds to the loss of water or M-HY.* Analogous peaks at M-32 for the loss of methanol from the molecular ion are observed in the spectra of I and III–VI which contain a OMe instead of OH at position 9a (Table 1). Thus, mass spectrometry may be used to determine the nature of the substituent at position 9a.

* Metastable ions (m*) are indicated in Table 2 as they were observed in the spectra.

In the spectrum of II the next fragment ion (*m/e* 316) corresponds to loss of a Me radical from the M-H_2O ion. This ion is shifted from M-33 to M-47 in all samples which contain both X = Y = OMe. In the mitomycin compounds (III, IV) containing an amino group (X = NH_2) at position 7, the M-HY-Me ion is not observed. The M-47 ion was not shifted to M-50 in VI (Z = CD_3), thus the Me in the OMe substituent in the X = OMe mitomycin compounds appears to be the only Me group being lost. For structure determination the presence of a M-HY-Me ion in the mass spectrum of an unknown mitomycin may be used as a diagnostic test for the presence of a OMe at position 7.

Many fragment ions in the spectra of the mitomycins arise from fragmentation of the carbamate ester group. The highest mass ion in this regard was very weak in the spectra of the parent compounds I–IV and corresponds to loss of a HNCO unit from $M^{+\cdot}$. This ion was observed in II at *m/e* 306. The M-H_2NCO_2H ion, **a**, (*m/e* 288 in II), however, was an intense ion in the spectra of all the compounds studied except VII. Ion **a** undergoes further fragmentation as indicated by metastable ions (Table 2) to give other ions. Loss of a Me was observed at *m/e* 273 in II and was an intense ion in all other spectra also. The Me group lost in the **a**-Me ion is not definitely established by the spectra. However, examination of the spectra does limit the number of possibilities. There was no indication in the spectrum of VI, Z = CD_3, of an ion corresponding to **a**-CD_3. Thus position 1a does not seem to be contributing to the **a**-Me ion. In VIII the **a**-Me is observed but this compound does not contain a Me at X or Y. This would suggest the source of the Me is likely either the C-6 Me or the C-10 group following possible hydrogen rearrangement.



Scheme 1

Ion **a** underwent another type of fragmentation shown in Scheme 1 in which the Y substituent was lost to yield **b** or **b'** (*m/e* 271 in II). However, the presence of a *m/e* 257 ion (M-H_3NCO_2-MeO) in the spectrum of II where Y = OH indicates that **b** and **b'** may not be the only structures which may be invoked to account for the M-H_3NCO_2-MeO ions in the spectra of I, III–VI. Ions corresponding to the loss of other small functional groups from **a** or **a**-CH_3 are tabulated in Table 1. For example, in the

TABLE 1. CORRESPONDING IONS IN THE MITOMYCIN SPECTRA

m/e
Composition
(Rel. Abund.)

	I	II	III	IV	V	VI	VII	VIII
$M^{+\cdot}$	349 $C_{16}H_{19}N_3O_6$ (1)	349 $C_{16}H_{19}N_3O_6$ (2)	334 $C_{15}H_{18}N_4O_5$ (1)	348 $C_{16}H_{20}N_4O_5$ (1)	363 $C_{17}H_{21}N_3O_6$ (0·4)	366 $C_{17}H_{18}D_3N_3O_6$ (1)	331 $C_{16}H_{17}N_3O_5$ (40)	316 $C_{15}H_{16}N_4O_4$ (73)
$[M\text{-}HY]^{+\cdot}$	317 $C_{15}H_{15}N_3O_5$ (7)	331 $C_{16}H_{17}N_3O_6$ (3·5)	302 $C_{14}H_{14}N_4O_4$ (10)	316 $C_{15}H_{16}N_4O_4$ (14)	331 $C_{16}H_{17}N_3O_5$ (6)	334 $C_{16}H_{14}D_3N_3O_5$ (14)	—	—
$[M\text{-}HY\text{-}CH_3]^+$	302 $C_{14}H_{12}N_3O_5$ (8)	316 $C_{15}H_{14}N_3O_5$ (4)	—	—	316 $C_{15}H_{14}N_3O_5$ (10)	319 $C_{15}H_{11}D_3N_3O_5$ (20)	—	—
$[M\text{-}HNCO]^{+\cdot}$	306 $C_{15}H_{18}N_2O_5$ (0·5)	306 $C_{15}H_{18}N_2O_5$ (1)	291 $C_{14}H_{17}N_3O_4$ (1)	305 $C_{15}H_{19}N_3O_4$ (0·6)	320 $C_{16}H_{20}N_2O_5$ (0·3)	323 $C_{16}H_{17}D_3N_2O_5$ (0·5)	288 $C_{15}H_{16}N_2O_4$ (14)	273 $C_{14}H_{15}N_3O_5$ (40)
$[a]^{+\cdot}$ or $([M]^{+\cdot}\text{-}H_3CO_2N)$	288 $C_{15}H_{16}N_2O_4$ (50)	288 $C_{15}H_{16}N_2O_4$ (51)	273 $C_{14}H_{15}N_3O_3$ (65)	287 $C_{15}H_{17}N_3O_3$ (51)	302 $C_{16}H_{18}N_2O_4$ (29)	305 $C_{16}H_{15}D_3N_2O_4$ (70)	270 $C_{15}H_{14}N_2O_3$ (8)	255 $C_{14}H_{13}N_3O_2$ (60)
$[a\text{-}CH_3]^+$	273 $C_{14}H_{13}N_2O_4$ (10)	273 $C_{14}H_{13}N_2O_4$ (16)	258 $C_{13}H_{12}N_3O_3$ (19)	272 $C_{14}H_{14}N_3O_3$ (59)	287 $C_{15}H_{15}N_2O_4$ (40)	290 $C_{15}H_{12}D_3N_2O_4$ (92)	255 $C_{14}H_{11}N_2O_3$ (10)	240 $C_{13}H_{10}N_3O_2$ (27)
$[b]^+$ or $([M]^{+\cdot}\text{-}H_3CO_2N\text{-}Y)$	257 $C_{14}H_{13}N_2O_3$ (23)	271 $C_{15}H_{15}N_2O_3$ (7)	242 $C_{13}H_{12}N_3O_2$ (100)	256 $C_{14}H_{14}N_3O_2$ (60)	271 $C_{15}H_{15}N_2O_3$ (16)	274 $C_{15}H_{12}D_3N_2O_3$ (35)	—	—

	256	256	241	255	270	273		
$[a\text{-}CH_3OH]^{+\cdot}$	$C_{14}H_{12}N_2O_3$ (5)	$C_{14}H_{12}N_2O_4$ (3)	$C_{13}H_{11}N_3O_2$ (22)	$C_{14}H_{13}N_3O_2$ (20)	$C_{15}H_{14}N_2O_3$ (4)	$C_{15}H_{11}D_3N_2O_3$ (13)	—	—
	245	245	230	244	259	262	227	
$[a\text{-}CH_3\text{—}CO]^+$	$C_{13}H_{13}N_2O_3$ (5)	$C_{13}H_{13}N_2O_3$ (8)	$C_{12}H_{12}N_3O_2$ (12)	$C_{13}H_{14}N_3O_2$ (12)	$C_{14}H_{15}N_2O_3$ (7)	$C_{14}H_{12}D_3N_2O_3$ (11)	$C_{13}H_{11}N_2O_2$ (8)	—
	243	243	228	242	257	260	225	
$[a\text{-}CH_3\text{—}CH_2O]^+$	$C_{13}H_{11}N_2O$ (10)	$C_{13}H_{11}N_2O_3$ (8)	$C_{12}H_{10}N_3O_2$ (11)	$C_{13}H_{12}N_3O_2$ (12)	$C_{14}H_{13}N_2O_3$ (9)	$C_{14}H_{10}D_3N_2O_3$ (16)	$C_{13}H_9N_2O_2$ (2)	—
		70		70	70	73		
$[c]^+$	—	C_4H_8N (100)	—	C_4H_8N (25)	C_4H_8N (24)	$C_4H_5D_3N$ (25)	—	—
	54	68	54	68	68	71		
$[d]^+$	C_3H_4N (7)	C_4H_6N (12)	C_3H_4N (10)	C_4H_6N (41)	C_4H_6N (35)	$C_4H_3D_3N$ (45)	—	—
	28	42	28	42	42	45	42	42
$[e]^+$	CH_2N (<50)*	C_2H_4N (23)	CH_2N (<18)*	C_2H_4N (100)	C_2H_4N (100)	$C_2H_1D_3N$ (100)	C_2H_4N (19)	C_2H_4N (21)

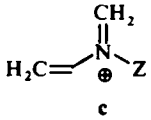
* The $CO:CH_2N$ ratio varied from <1 to >1 as the sample was exposed over ~10 min at the vaporization temp.

spectrum of II ions are observed at *m/e* 256 (**a**-CH_3-OH), *m/e* 245 (**a**-CH_3-CO) and *m/e* 243 (**a**-CH_3-CH_2O),

The *m/e* 44 ion was observed as an intense ion in the spectra of all the naturally occurring mitomycins and its composition CH_2NO indicates that this is the carbamylium ion. The *m/e* 44 ion was considerably weaker in the spectrum of V than in the other spectra.

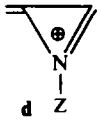
The base peak in the spectrum of II occurred at *m/e* 70 (C_4H_8N). This was also a prominent ion in the spectra of IV and V. This C_4H_8N unit may be readily accounted for by fragmentation of II to yield an ion, **c**, containing C atoms 1, 1a, 2 and 3 in addition to the aziridine ring nitrogen. In order to test this proposal the spectrum of VI, the CD_3 analog of V, was obtained. The spectrum of VI (Fig. 6) contained a prominent ion at *m/e* 73 ($C_4H_5D_3N$). Thus the 1a Me is indicated to be retained in the *m/e* 70 ion in the spectra of II, IV and V. Interestingly, ion **c** is not observed in the spectra of I and III at either *m/e* 70 or *m/e* 56 as a lower homologue (**c**, Z = H).

Another strong ion, **d**, at *m/e* 68 (C_4H_6N) was observed in the spectra of II, IV and V. The homologous ion at *m/e* 54 (C_3H_4N) was observed in the spectrum of I and III. Ion **d** underwent a shift of three mass units to *m/e* 71 in the spectrum of VI. Thus, hydrogen transfer from position 1, 2 or 3 to the indole-benzoquinone system is indicated for its formation. A possible structure for ion **d** consistent with the available data is shown below.



c

Z = Me or CD_3



d

Z = Me, H, CD_3

Ion **e** at *m/e* 42 was the base peak in the spectra of IV and V, and an intense ion in the spectra of II, VII and VIII. This ion was shifted to *m/e* 28 in the spectra of I and III.* In addition, it was shifted to *m/e* 45 in the spectrum of VI where it was also the base peak. The shift of this ion by three mass units in the trideutero compound implies that the structure retains completely the position 1a Me group on the aziridine nitrogen and points to a Me-$N^{+}\equiv$CH structure.

Thus, mass spectrometry provides a means for determining the nature of the Z substituent on the aziridine nitrogen in an unknown mitomycin. Ions **c**, **d** and **e** will occur, respectively, at *m/e* 70, 68 and 42 when Z = Me. However, when Z = H ions **d** and **e** are shifted to *m/e* 54 and 28, respectively, and ion **c** does not appear.

A comparison of the spectra of IV and V allows one to observe the effect of varying the X substituent between —NH_2 and —OMe. As previously noted the major difference between the two types of compounds in the high mass region is that only the X = OMe compounds fragment *via* loss of HY followed by loss of a Me group. A strong ion at *m/e* 273 occurs in the spectrum of V, but it is not observed in IV or in other homologous mitomycins with X-OMe. This ion has a composition $C_{14}H_{13}N_2O_4$ which is consistent with the loss of MeOH, HNCO, and Me· from the molecular ion. In the spectrum of VI this ion retains all three D atoms and occurs at *m/e* 276. Thus,

* m/e 28 in these spectra also contained considerable amounts of CO which also arose from the sample.

TABLE 2. METASTABLE IONS

	Found (Calcd)							
	I	II	III	IV	V	VI	VII	VIII
$M^{+\cdot} \rightarrow M\text{-}HY^{+\cdot}$	288·0 (287·9)	—	272·9 (273·1)	286·9 (286·9)	302·0 (301·8)	304·8 (304·8)	—	—
$M\text{-}HY^{+\cdot} \rightarrow M\text{-}HY\text{-}CH_3{}^{+\cdot}$	288·0 (287·7)	301·8 (301·7)	—	—	302·0 (301·7)	304·8 (304·7)	—	—
$M^{+\cdot} \rightarrow M\text{-}HNCO^{+\cdot}$	—	268·4 (268·3)	—	—	—	—	250·8 (250·6)	235·9 (235·8)
$M^{+\cdot} \rightarrow a^{+\cdot}$	237·5 (237·7)	237·5 (237·7)	223·0 (223·1)	236·8 (236·7)	251·2 (251·3)	254·1 (254·2)	—	205·9 (205·8)
$a^{+\cdot} \rightarrow a\text{-}Me^{+}$	258·9 (258·8)	258·9 (258·8)	244·0 (243·8)	258·0 (257·8)	272·8 (272·7)	—	—	—
$a^{+\cdot} \rightarrow a\text{-}OMe^{+}$	229·4 (229·3)	229·5 (229·3)	214·6 (214·5)	228·3 (228·3)	243·3 (243·2)	275·8 (275·7)	—	—
$M\text{-}HY\text{-}Me^{+} \rightarrow$ $M\text{-}HY\text{-}Me\text{—}HNCO^{+}$	222·0 (222·1)	—	—	—	—	—	—	—
$M^{+\cdot} \rightarrow M\text{-}CH_2NO^{+}$	—	—	—	—	—	—	248·9 (248·8)	234·0 (234·1)
$M\text{-}HNCO^{+\cdot} \rightarrow$ $M\text{-}HNCO\text{-}Me^{+}$	—	—	—	—	—	—	258·7 (258·8)	244·0 (243·8)

the 1a-Me is retained in this structure. It is not clear why this ion should be so prominent in the spectrum of V and not occur in the spectra of I–IV.

In the spectrum of IV a prominent ion at *m/e* 203 occurs which is a doublet, **f** and **f'**, of nearly equal intensity with compositions $C_{11}H_{11}N_2O_2$ and $C_{10}H_7N_2O_3$, respectively. In the spectrum of III the same doublet occurs, but this ion is shifted in the spectra of II, V, and VI to *m/e* 218 ($C_{12}H_{12}N_1O_3$ and $C_{11}H_8N_1O_4$) corresponding to X = OMe rather than —NH_2. Since the ion is unshifted in VI, the 1a Me group and possibly larger portions of the aziridine ring are lost during formation of this ion.

Although **f** and **f'** are helpful in determining the nature of the X substituents, these ions are not always prominent (absent in I) and their usefulness for unknown structure determinations is limited. The most consistent fragmentation for this purpose appears to be the M-HY-Me sequence.

It is interesting to note that typical benzoquinone fragmentations[10] involving in the mitomycins loss of carbons 6, 7 and 8 with their substituents are not observed for either type (X = NH_2 or X = OMe) of mitomycin.

The two members of the anhydro-*apo* class of mitomycins show similar spectra as that of their naturally occurring precursors, but the intensities of the high mass ions are greatly increased. The molecular ions are much more intense as are other ions such as the M-HNCO fragment ions (*m/e* 288 in VII and *m/e* 273 in VIII). Ions at *m/e* 273 in VII and *m/e* 258 in VIII correspond to M-HNCO-Me. In this class of compound loss of $H_2NCO\cdot$ from $M^{+\cdot}$ is a favorable cleavage as shown by the ions at *m/e* 287 in VII and *m/e* 272 in VIII. Once again only the compound VII (X = OCH_3) shows a significant M-Me ion (*m/e* 316). However, ion **a** is much weaker in VII (*m/e* 270) than in VIII (X = NH_2) at *m/e* 255. Other ions corresponding to **a**-Me and fragment ions from **a** are indicated in Table 1.

It is also interesting to note that both VII and VIII are structurally capable of producing both ions **c** and **d**, but neither of them did so. In addition ion **e** was not intense in either spectrum.

In conclusion, we feel that this study shows that mass spectrometry provides a means for readily obtaining structural information and identifying new members of the mitomycin family such as mitiromycin[3] and the new antibiotics reported by Nomura *et al.*[7]

EXPERIMENTAL

Mass spectra were determined by means of an AEI MS-9 mass spectrometer at an electron voltage of 70 eV. Accurate mass measurements were carried out (within 3 mmu of the calculated values) at a resolving power ca. 10,000 on either an AEI MS-9 or CEC 21-110 instrument. The direct insertion inlet technique was used at a temp of 180–220°.

N-Methyl mitomycin A (V). A mixture of DMF (1·25 ml) water (1·25 ml) and $NaHCO_3$ (41·5 mg) was treated with 10 mg mitomycin A and 0·5 ml MeI. The mixture was stirred in a closed container for several hr and then allowed to stand overnight at room temp. After aerating with N_2 to remove excess MeI, the mixture was evaporated to dryness *in vacuo* and the residue was extracted with $CHCl_3$. The $CHCl_3$ was evaporated to about 0·5 ml; careful addition of light petroleum gave a partially crystalline ppt (10·4 mg). This was further purified by partition chromatography on a Celite* support using solvent system heptane: EtOAc:MeOH:water (60:40:17:4). The fraction coming off the column at about 3. holdback volumes was collected, isolated, and recrystallized from CCl_4 and light petroleum, yield about 6 mg.

* Trademark for diatomaceous earth, product of Johns Mansville.

N-*Trideuteromethyl mitomycin* A (VI). The above preparation of V was repeated using 99·8% pure trideuteromethyl iodide.

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