M. T. Bowers, Ed., Academic Press, New York, 1979, Chapter 11.
(52) G. I. MacKay, R. S. Hemsworth, and D. K. Bohme, *Can. J. Chem.*, **54**, 1624 (1976).
(53) J. H. Richardson, L. M. Stephenson, and J. I. Brauman, *J. Am. Chem. Soc.*, **97**, 2967 (1975).
(54) R. J. W. LeFevre, *Adv. Phys. Org. Chem.* **3**, 1 (1965). The bulk polarizability is taken as a qualitative approximation to the microscopic polarizability; ref 8.
(55) R. J. Irving, L. Nelander, and I. Wadso, *Acta. Chem. Scand.*, **18**, 769 (1964).
(56) A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases", Wiley, New York, 1962.

Alkylation Reactions of Mitomycin C at Acid pH

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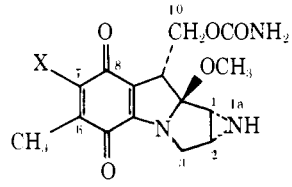
Abstract: Mitomycin C readily alkylates inorganic phosphate and the phosphate group of various nucleotides in aqueous solution at acid pH. The products of this type of reaction are 2,7-diaminomitosenes containing a phosphate group in the 1-position. The reaction is appreciable only below pH 5, requiring protonation of the aziridine ring of mitomycin C ($pK_a' = 4$). The specific reactions studied were the following: alkylation of inorganic phosphate yields 1,2-*cis*- and -*trans*-2,7-diaminomitosene 1-phosphate, in greater than 9/1 ratio. Alkylation of 5′-uridylic acid results in *cis*- and *trans*-2,7-diaminomitosene 1-(5′-uridylate), in approximately 4/1 ratio. 5′-Uridine triphosphate is alkylated at its terminal phosphate group, to give the corresponding 1-substituted 2,7-diaminomitosene, also with predominant cis composition. The structure of these products was proven by quantitative phosphate analysis, ultraviolet spectra, and enzymatic degradation into known products. UpU yields a small amount of a mitosene adduct which was not characterized. Uridine itself is not alkylated by mitomycin C. Hydrolytic ring opening of the protonated aziridine ring of mitomycin C competes with the phosphate alkylation reactions, yielding *cis*- and *trans*-2,7-diamino-1-hydroxymitosenes. The phosphate compounds described represent the first characterized examples of alkylation of nucleotides by mitomycin C, supporting the previous hypothesis that the mitomycins are biological alkylating agents.

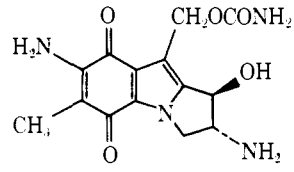
Mitomycin C (**1a**; MC), the potent antibiotic and clinically useful antitumor agent,[1-3] presents an interesting challenge to correlate chemical behavior and biological activity. It contains an aziridine ring, rare in natural products, and the well-known antitumor activity of various synthetic aziridines[4a] led early to the suggestion that the aziridine ring of MC is involved in its mechanism of action.[5] Since aziridines, including the class of N-mustards, where the aziridine form is the reactive tautomer,[6a] are powerful alkylating agents, MC was predicted to alkylate its biological target, most likely DNA, analogously.[5,7] Consistent with this hypothesis, DNA isolated from MC-treated bacteria contained covalently bound MC[8] and, in addition, its two complementary strands were cross-linked,[9,10] indicating two binding functions of MC to DNA rather than one. The cross-linking action was considered to be the direct cause of the cytotoxicity of the drug,[9] although more recently the monofunctional attachment which predominates 10- to 20-fold over the number of cross-links[8,11] has also been implicated as biologically significant damage to DNA.[12-14]

In the absence of cells, the DNA-binding and cross-linking effects of MC could only be demonstrated if a reducing agent (chemical or enzymatic) was also added, indicating that the active form of the drug is generated in the cell by reduction.[5,7] More recently, it was shown that low pH (pH 4) alone also activates MC to bind and cross-link DNA in vitro.[15]

A detailed chemical hypothesis was advanced by Iyer and Szybalski[7] for the functioning of MC as a reductively activated bifunctional alkylating agent and this mechanism was further refined recently by Moore.[16] Despite this apparent interest in the molecular mode of action of MC, experimental verification of the proposed chemistry is lacking. Alkylation reactions of the mitomycins have not been characterized, the redox chemistry of MC itself is complex and not well understood,[17,18] and efforts to isolate and characterize MC-nucleotide adducts from model reactions or from MC-nucleic acid complexes have been unsuccessful so far.[19] The only product ever characterized from any reaction of reduced MC is the bisulfite adduct **5**.[18]



1a, X = NH_2
b, X = OCH_3



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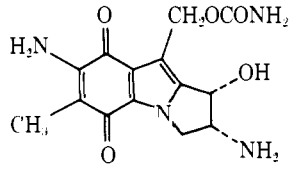

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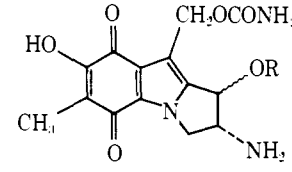
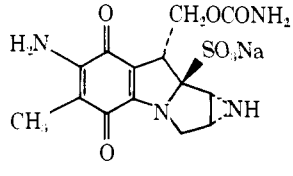
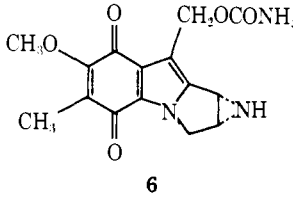


4a, R = H
b, R = PO_3H_2



5



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Its mechanism of formation and relevance to the DNA-binding and cross-linking action of reduced MC is unknown. We undertook the task to seek basic evidence for the postulated alkylating properties of MC. As our first approach, we succeeded in observing the alkylation of a series of phosphate compounds by MC under the simple low pH activation conditions.

Experimental Section

Materials. The materials used and their sources are as follows: mitomycin C, Bristol Laboratories, Syracuse, N.Y.; bacterial alkaline phosphatase, snake venom phosphodiesterase, Worthington Biochem. Corp., Freehold, N.J.; nucleotide pyrophosphatase (type III, from

0002-7863/79/1501-6063$01.00/0

Table I. Analytical Properties of New MC Derivatives and Parent Compounds

compound	elution vol, mL[a]	R_f[b] A	B	C	D	E	electrophoretic mobility[c] F	G	H	λ_{max}, nm ($\epsilon \times 10^{-3}$) [d]
1a	96	0.88	0.80		0.75					367, 21.8 (in H_2O)[21]
2	185	0.95	0.53	0.27	0.85	0.60	−3.3	−1.6	0	312, 11.1; 248,
3	.206	0.94	0.43	0.20	0.85	0.60	−4.5	−1.6	0	17.1[e]
4a (trans)	127	0.94		0.40			−3.1	+0.7		346, 3.92; 294, 15.9;
4a (cis)	139	0.94		0.32			−4.5	+0.7		235, 21.7 (in 0.1 N HCl)[20]
4b	98	0.53								
7b	60	0.32			0.48	0.10		+17.0		262, 10.0[38]
7c	52	0.1			0.17			+15.5		262, 10.0[38]
8a	130	0.53			0.66	0.15	0	+5.6	+11.8	310, 13.14; 248, 21.74
8b	100	0.65			0.64	0.23	0	+1.3	+11.3	312, 12.2; 252, 26.64
8c	74	0.1			0.21			+11.5		312, 13.2; 252, 27.98

[a] Column = 1.5 × 41.5 cm Sephadex G-25 (fine). Buffer: 0.02 M NH_4HCO_3. The numbers represent the peak elution volumes. Blue dextran (void volume) and NaCl (inclusion volume) are eluted at 33 and 67 mL, respectively. [b] Systems A, B: TLC plates (silica, Macherey-Nagel); (A) isopropyl alcohol–1% NH_4OH, 2:1 (v/v); (B) methanol. Systems C, D: TLC plates (cellulose, Eastman); (C) isopropyl alcohol–concentrated HCl–H_2O, 68:17:15 (v/v/v); (D) isobutyric acid–0,5 M NH_4OH, 10:6 (v/v). System E: paper (Whatman 40, descending); *n*-propyl alcohol–1% NH_4OH, 2:1 (v/v). [c] Paper electrophoresis (Whatman 3 MM), 22 V/cm, 2 h; (F) 0.02 M sodium citrate buffer, pH 3.5; (G) 0.02 M sodium phosphate buffer, pH 7.2; (H) 0.02 M sodium borate buffer, pH 10.0. [d] In 0.02 M sodium phosphate buffer, pH 7.5, unless otherwise indicated. [e] Determined by quantitative comparison with the known spectra in methanol.[21]

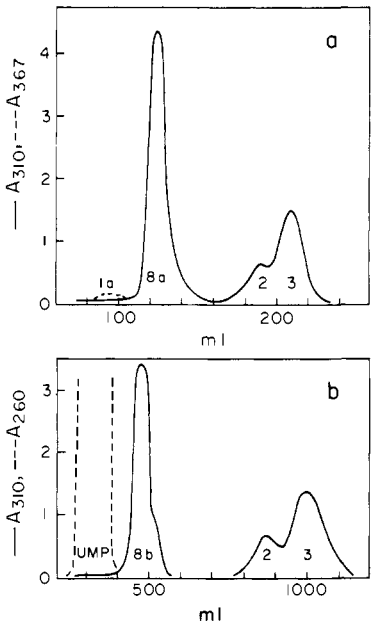


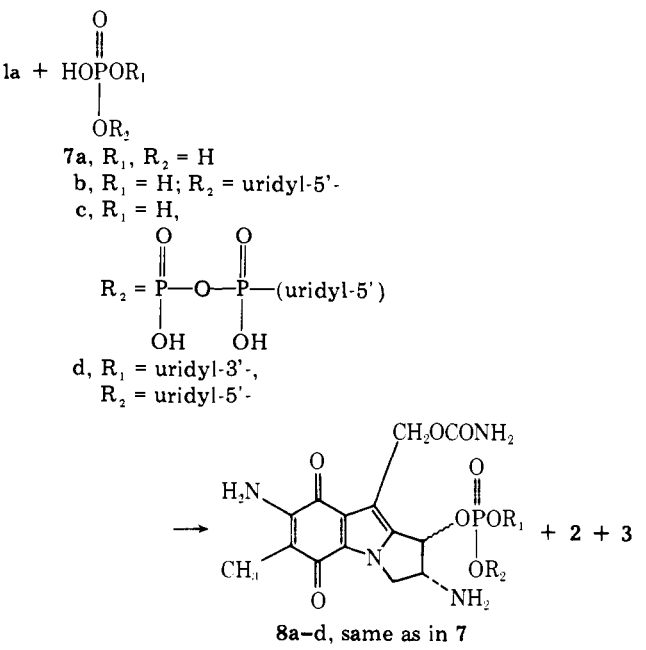
Figure 1. Separation of alkylation and hydrolysis products of MC on Sephadex G-25 columns. (a) Products from the reaction with inorganic phosphate. Separation conditions are given in Table I. (b) Products from the reaction with 5′-UMP (**7b**). Separation conditions are the same as in Table I except for the column size (3.7 × 36 cm).

Crotalus atrox venom), and uridine 5′-triphosphate (5′-UTP; type III, from yeast), Sigma Corp., St. Louis, Mo.; uridine 5′-monophosphate (5′-UMP) and uridylyl(3′ → 5′)uridine (UpU), Boehringer Mannheim Corp., Indianapolis, Ind.; DEAE-cellulose (Cellex D) and AG-50W-X-8 (H^+ form), 200–400 mesh cation-exchange resin, Bio-Rad Corp., Richmond, Calif.

Methods: Alkylation reactions of MC (1a) with phosphate compounds (7) (see Scheme I) were conducted under the following standard conditions: An aqueous solution of the phosphate compound was acidified to pH 3.0 and the concentration was adjusted to 0.34 M. In the case of **7a**, acidification was accomplished by adding dilute NaOH to aqueous H_3PO_4 and, in cases **7b–d**, by adding beads of AG-50W-X-8 (H^+ form) cation-exchange resin to an aqueous solution of the NH_4^+-salt form of the respective nucleotide. Mitomycin C (0.0026 M) was added to the acidic phosphate or nucleotide solution. The reaction volume was usually 1.5 mL. The mixture was incubated at 25 °C for 2 h under stirring, then neutralized by the addition of dilute NaOH.

Separation, Isolation, Identification, and Quantitation of the

Scheme 1



Products. The above reaction mixture was submitted to gel filtration chromatography on a Sephadex G-25 column. (See Table I for standard conditions.) In the chromatographic elution patterns (e.g., Figure 1) mitosene[20] type products could be distinguished from nucleotides by absorbance at 310 nm, a characteristic approximate absorption maximum of the 7-aminomitosene chromophore.[21] (Compounds **2** and **3** exemplify mitosene derivatives while **1a** and **1b** are mitosanes.) The purple color of mitosenes, easily seen on TLC plates or paper, gives similar indications. For analytical purity, the alkylation products were rechromatographed once or more. Desalting of the column fractions was accomplished by evaporation of the NH_4HCO_3 at 37 °C in vacuo. Quantitative analysis of all products was based on UV spectrophotometry in aqueous media (Table I) by using a Gilford Model 250 spectrophotometer. Other techniques will be specified in the Results section.

Results

Upon chromatographic separation of the reaction mixtures, excess unalkylated nucleotide was eluted first, followed by its product of alkylation by MC and, finally, the pair of peaks of the isomeric hydrolysis products of MC (**2** and **3**) (Figure 1). Some unreacted MC (5–7% of the starting material) was also

eluted usually, well separated from the products except from **8b** (Table I). In this latter case, the standard reaction conditions were modified to 4-h incubation instead of 2 h, resulting in complete elimination of unreacted MC. Identification of each of these substances was carried out as follows:

Unchanged nucleotides (7a–d) and MC were recognized by their elution volumes, R_fs, and electrophoretic mobilities (Table I).

MC hydrolysis products 2 and 3 were identified by the identity of their elution volumes, R_fs, and electrophoretic mobilities in several systems (Table I) with those of authentic **2** and **3** which were prepared by the procedure of Stevens et al.[21] and separated as described by Taylor and Remers.[22] The molar ratios of **2** to **3** were 1 to 3 (± 0.2), either when formed as byproducts of the MC alkylation reactions or when prepared by the published procedures.

Alkylation Products. (a) The alkylation product of inorganic phosphate (**8a**) was eluted as a single peak which also showed homogeneity on TLC and electrophoresis (Table I) and it migrated as an anion in the latter system at neutral and alkaline pH while it had no charge at pH 3.5.[23] It was retained on a DEAE-cellulose anion-exchange column when the column was washed with 0.02 M NH_4HCO_3 and was eluted as a single peak with 0.2 M NH_4HCO_3. Its ultraviolet spectrum was very similar to that of **2** or **3.** Phosphate analysis[24] gave 1.0 μmol of phosphate per 13.4 A_{310} units of **8a,** indicating clearly one phosphate group per mitosene chromophore. The ε values (Table I) are based on the phosphate analysis. Treatment of **8a** with alkaline phosphatase (1.67 μmol of **8a** in 2.0 mL of 0.01 M Tris–0.002 M $MgCl_2$, pH 8.5, 17.8 units of enzyme; 37 °C, 1.5 h) yielded stoichiometric amounts of inorganic phosphate, determined by analysis,[25] and **2** and **3** were determined by the standard Sephadex G-25 column separation, in <5% and 92% yields, respectively. Control incubation without the enzyme gave unchanged starting material.

These results prove the structure of **8a** and indicate that alkylation of inorganic phosphate gives almost exclusively the 1,2-cis isomer.

(b) The alkylation product of 5′-UMP (**8b**) was eluted as a main peak with a shoulder (Figure 1b). On repeated chromatography, the two components (major and minor) could be completely separated and were shown to be the 1,2-cis and 1,2-trans isomers of **8b,** respectively, as follows: The two substances had identical properties on TLC and electrophoresis, migrating on the latter as anions at neutral and alkaline pHs but were uncharged at pH 3.5[23] (Table I). The unexpectedly high mobility at pH 10.0 (as high as that of **8a** which has two negative charges on its phosphate) is undoubtedly due to the formation of a negatively charged borate complex of the uridine unit with the borate buffer, a phenomenon well-known in nucleotide electrophoresis.[26] Both isomers of **8b** had identical UV spectra which were a composite of the uridine and mitosene chromophores. Phosphate analysis[24] indicated 1.0 μmol of phosphate per 12.2 A_{313} units, i.e., per 1 μmol of mitosene. ε values are given in Table I, based on 1 mol of phosphate. Both isomers of **8b** were resistant to alkaline phosphatase. Snake venom diesterase digestion (1 μmol of **8b** (cis or trans) in 1 mL of 0.005 M Tris–0.001 M $MgCl_2$, pH. 8.5, 15 μg of enzyme; 37 °C, 3 h), however, hydrolyzed each to 5′-UMP and a mitosene: **3** from the major and **2** from the minor **8b** isomers. When an unseparated mixture of **8b** isomers was digested with snake venom diesterase, stoichiometric amounts of 5′-UMP and a mixture of **2** and **3** were produced in a molar ratio of **2**/**3** = ¼. These results show evidence for the structure of **8b** and indicate that alkylation of 5′-UMP gives a mixture of the 1,2-trans and 1,2-cis isomers, the latter predominating.

(c) The alkylation product of 5′-UTP (**8c**) was eluted from Sephadex G-25 as one peak. Several rechromatographies were necessary to remove all 5′-UTP from the analytical sample which was homogeneous on TLC and electrophoresis (Table I), migrating in the latter somewhat slower than UTP at neutral pH. Its UV spectrum was identical with that of **8b.** Analysis of total phosphate indicated 3.0 μmol of phosphate per 13.2 A_{312} units, i.e., per μmol of mitosene chromophore. ε values are given in Table I, based on 3 mol of phosphate.

Hydrolysis of **8c** in 1 N HCl (100 °C, 2 h) released 2.0 mol of P_i per mol of **8c,** the same as from control UTP itself. This was first unexpected since, under these conditions, γ-alkylated ATP was reported to give only 1 mol of P_i, originating from the β-phosphate, while the γ-phosphate retained its alkyl group.[27] Since in our case, the corresponding alkyl γ-phosphate is **8a,** we tested its stability under these conditions and found it to be completely hydrolyzed to P_i and **2** or **3.** This explains the above results of the hydrolysis of **8c,** rendering them consistent with the structure (although not proving it, see Discussion). Treatment of **8c** with nucleotide pyrophosphatase from snake venom (0.17 μmol of **8c** in 0.05 mL of 0.02 M Tris (pH 7.5)–0.25 M $MgCl_2$, 1.1 enzyme units, 37 °C, 1 h[28]) did not result in any hydrolysis. This enzyme normally hydrolyzes proximal pyrophosphate bonds of nucleotides.[28] The resistance of **8c** may be due to the presence of the unusual (mitosene) structure in the substrate. These negative results leave the structure of **8c** without a rigorous proof. The mode of formation, chemistry of analogous reactions, together with the analytical data strongly suggest its correctness, however (see Discussion). Indication that **8c** as isolated is also a mixture of the 1,2 cis and trans isomers was obtained on chromatography over a longer (1.5 × 56 cm) Sephadex G-25 column, on which it separated as a peak with a shoulder much like **8b** in Figure 1b. The size ratio of the main peak to the partly overlapping minor peak could be roughly estimated as 7 to 1.

(d) The alkylation product of UpU (**8d**) was eluted from Sephadex G-25 as a small peak, partially separated from UpU and was identified only by its elution behavior (Table I) and the fact that it possessed the UV characteristics of mitosenes. Because of the extremely low yield (Table II), further characterization was not feasible.

(e) Uridine gave no alkylated product as tested by Sephadex G-25 chromatography, electrophoresis and several TLC systems.

Table II. Extent of Alkylation of Various Substrates by MC[a]

substrate	alkylated product	yield, %	1,2-cis/trans isomer ratio
7a	**8a**	56	>9
7b	**8b**	46	4
7c	**8c**	34	6
7d	unidentified	4	
uridine	none		

[a] Cf. Reaction Scheme I and Methods.

Yields of the alkylation products are given in Table II, based on mitomycin C and determined by UV spectrophotometry. Compounds **2** and **3** were the additional products in each case (1/3 ratio, see above). Overall recoveries of material were in the range of 92–104%.

pH Dependence of Alkylation of Inorganic Phosphate by MC. The effect of pH on the extent of conversion of MC to its hydrolysis (**2, 3**) and phosphate alkylation products (**8a**) is illustrated in Figure 2. The combined yield of these products increases with decreasing pH, resembling a base protonation curve with pK_a = 4.0. The yield curve for **8a** alone is similar until very low pH when it drops, while the yield of **2** and **3** (combined) increases. At pHs 1.33 and 2.05 we noted some hydrolysis of the 7-NH_2 group, by the appearance of the corresponding products from **2, 3** and **8a** (**4a,b**). The yields of **2, 3,** and **8a** include these 7-OH analogues since they were un-

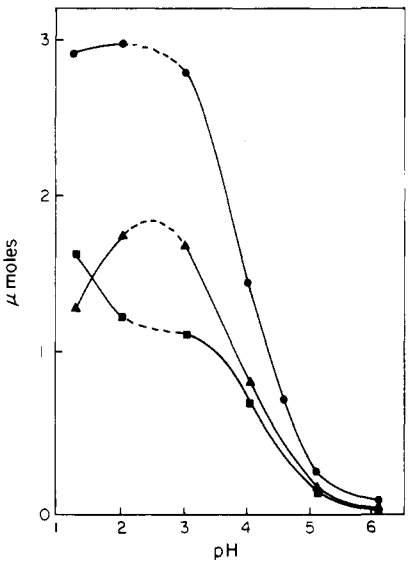


Figure 2. pH dependence of alkylation of inorganic phosphate by MC. MC and inorganic phosphate were incubated under the standard reaction conditions (Methods), except for the variation of pH. The pH was varied by addition of dilute NaOH to H_3PO_4 solution to desired pH. The total reaction volume was 1.15 mL, corresponding to 3.0 μmol of MC, in each case. Products were analyzed as described under Methods. (●—●) Combined yield of alkylation and hydrolysis products from MC; (▲—▲) yield of alkylation products alone; (■—■) yield of hydrolysis products alone.

doubtedly formed in a secondary step, according to the well-known progressive stages of acid hydrolysis of MC.[21] Detection and identification of these substances was achieved as follows: **4b** was a new, early peak from the Sephadex G-25 column, showing the well-known spectral and indicator properties (purple in base, orange in acid) of 7-OH-mitosenes[20] and containing 1 mol of phosphate per 7-OH-mitosene chromophore. The same product also was formed when **8a** was incubated in 0.1 N HCl for 2 h as a control experiment. Compound **4a** was detected in the **8a** containing fraction of the column by comparison of its elution, TLC, and electrophoretic behavior with an authentic standard (Table I) and was quantitatively analyzed by elution of spots from the electrophoresis paper in 1 M NH_4OH and determining the eluates by spectrophotometry. The authentic standard **4a** was prepared by prolonged hydrolysis of MC in 0.1 N HCl. This procedure was previously described to give **4a** as a single product with unspecified stereochemistry.[20,21] Our Sephadex column as well as other systems (Table I) separated the crude product of hydrolysis into the 1,2-cis and 1,2-trans isomers of **4a** (3/1 molar ratio). The assignment of the stereochemistry of the two substances (Table I) was based upon our finding that in 0.1 N HCl **2** was hydrolyzed exclusively to one, consequently assigned trans, and **3** to the other, consequently assigned cis.

Attempted Alkylation of P_i with Mixed 2 and 3. Mixed **2** and **3** (ca. 1/3 ratio) were incubated with P_i at pH 3.0 under the standard reaction conditions, then analyzed as usual. Except for small amounts of **4a** and an unknown byproduct, **2** and **3** were recovered unchanged. The unknown byproduct did not contain phosphate as ascertained by total phosphate analysis and behaved as a cation on electrophoresis.

Discussion

Formation of the mitosene phosphate derivatives **8** demonstrates that MC is a functional alkylating agent under mild acid conditions. The sum of the yield of the phosphorolytic and hydrolytic reaction products depends on pH according to the shape of a protonation curve with $pK_a' = 4.0$ (Figure 2). This indicates that protonation of the aziridine ring (reported $pK_a' = 3.2$[29]) is required in accord with the mechanism of ring opening reactions of basic aziridines in general.[4b] Since the aziridine of the mitomycins is an unusually weak base (pK_a' of MC 3.2,[21] pK_a' of mitomycin B 4.3[30]) compared with simpler aziridines,[4c] evidently the lack of protonation under physiological conditions preserves this functional group in the mitomycins

It is now shown, however, that at lower pH the alkylating activity of MC is unmasked. The observed binding to DNA at pH 4 in vitro[15,30] is explained most likely by this mechanism, although the nucleophiles in DNA are not necessarily phosphate groups.

The observed pH dependence under pseudo-first-order conditions (Figure 2) allows further conclusions about the reaction mechanism. The extent of the hydrolytic and the phosphorolytic opening of the aziridine ring both show the same pH profiles between pH 3 and 6, indicating competition between water and phosphate for the protonated aziridine. The attacking phosphate species is apparently $H_2PO_4^-$, as indicated by the observed decline of the relative yield of the phosphorolysis product below ca. pH 3 (pK_a of $H_2PO_4^- = 2.12$).

The acid-catalyzed conversion of MC to aziridine-opened mitosene derivatives is not a simple process since it involves simultaneous elimination of CH_3OH and aziridine ring opening. The mechanism of such solvolytic ring openings of MC was recently studied by Remers and co-workers.[22,31,32] They conclude that, in each case studied, the process involves attack in the 1-position and the product has predominantly 1,2-cis stereochemistry, e.g., the cis/trans ratio of hydrolysis products **3** and **2** is 3/1. This is in contrast to the exclusively trans opening of simple aziridines.[4d] The participation of the neighboring methoxy group as a directing influence during the ring-opening step is not a likely explanation since the mitosene-type aziridine (**6**) also hydrolyzes predominantly to the cis aminohydrin and thus the problem of mechanism remains open.[32] In the present work ring-opening by inorganic phosphate gives almost exclusively the cis product (less than 5% trans product was detected, see Results) and the other phosphate-opened products **8b** and **8c** have cis/trans ratios ca. 4/1 and 9/1, respectively. Explanation for the greater cis/trans ratio of the phosphorolytic as compared with the solvolytic reactions must await the elucidation of the mechanism of these reactions.

Lack of Detection of a Second Alkylating Function of MC. Although hydrolytic displacement of the 7-amino group is appreciable at pH 2 and below as seen in our pH dependence experiments, no evidence for 7-phosphate type products was obtained in any of the reactions. Since recovery of material was at least 94% at all pHs tested, not much, if any, additional product could have remained undetected. In an independent experiment, a mixture of **2** and **3** was incubated with P_i under the standard conditions but no phosphate-containing product was detectable. This suggests that no second alkylating function of MC with reactivity comparable to that of the aziridine ring is present under these conditions. The second alkylating site implied indirectly by the acid-catalyzed cross-linking action of MC on DNA[15] is thus not revealed by these model experiments. Since the cross-links were reported to be "quite rare" compared with the monofunctional binding,[30] a model demonstration of the second site may be difficult because of low yields, although nucleophiles other than phosphate may be more reactive.

The series of nucleophiles **7a–d** chosen for this work represent basic types of cellular phosphate compounds (P_i, phosphomonoester, phosphodiester, pyrophosphate). Only reactions of the phosphate groups were observed, uridine itself did not react with MC. Alkylations of phosphates by ethylenimine and certain quaternary aziridines (reactive forms of N-mustards[6b])

have been well studied. Thus while inorganic phosphate[33,34] and monoalkyl phosphates,[34] including mononucleotides[35] are readily alkylated by aziridinium ring opening, dialkyl and diaryl phosphates are much less reactive.[34] The ring-opened products formed in the latter case have a tendency to reclose, and the isolated product is the aziridinium salt of the phosphate compound.[36] Our results conform to these principles: we obtained good and comparable yields of MC-alkylated products of P_i and 5′-UMP while very little if any of UpU (Table II). The small amount of product of the latter may be ionic association between UpU and the protonated hydrolysis product **2** or **3** ($pK_a' = 6.5$[21]) rather than **8d**, in analogy to what is believed to be the case in general of basic alkylating agents interacting with internucleotide phosphate groups,[6b] but the product was not characterized any further.

The reaction with UTP (**7c**) requires some comment. The terminal phosphate of pyrophosphate type compounds is nucleophilic, since its alkylation has been observed in a number of cases. For example, ATP and CDP were alkylated at their terminal phosphate by ethylenoxide[27] and ethylenimine,[37] respectively. Thus although a rigorous degradative proof of structure is lacking for the observed product from **7c**, its properties, its mode of formation as well as the above precedents make **8c** the most likely structure.

In conclusion: Alkylation products of MC using biological model nucleophiles were characterized for the first time. The conditions of their formation strongly suggest that the same type of reaction is responsible for the observed acid-catalyzed binding of MC to DNA in vitro,[15] although the nucleophiles are probably the bases rather than the phosphate groups. Activation of MC by acid might have significance in vivo, according to the quoted authors, since the known lowered pH of tumor tissues (e.g., ref 39) may promote acid-catalyzed binding and crosslinking of DNA by MC in such cells, contributing to the antitumor specificity of the drug. We may add that the fact that MC has proven especially effective against stomach cancers[3] may be related to acid-activated alkylation at the low pH of the gastric tract.

The methods and compounds described here could also serve as a model for exploring the long-postulated reductive alkylating properties of MC. Our finding that simple gel filtration gives good resolution and recovery of a large variety of MC derivatives should prove especially useful, in view of the reputed complexity of the redox chemistry of MC.

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References and Notes

(1) Wakaki, S.; Marumo, H.; Tomioka, K.; Shimizu, G.; Kato, E.; Kamada, H.; Kudo, S.; Fujimoto, Y. *Antibiot. Chemother.* **1958**, *8*, 228.
(2) Lefemine, D. V.; Dann, M.; Barbatschi, F.; Hausmann, W. K.; Zbinovsky, V.; Monnikendam, P.; Adam, J.; Bohonos, N. *J. Am. Chem. Soc.* **1962**, *84*, 3184.
(3) Crooke, S. T.; Bradner, W. T. *Cancer Treat. Rev.* **1976**, *3*, 121.
(4) Dermer, O. C.; Ham, G. E. "Ethyleneimines and Other Aziridines", Academic Press: New York, 1969; (a) p 425; (b) p 206; (c) p 108; (d) p 208.
(5) Schwartz, H. S.; Sodergren, J. E.; Philips, F. S. *Science* **1963**, *142*, 1181.
(6) Lawley, P. D. *Prog. Nucleic Acid Res. Mol. Biol.* **1966**, *5* (a) p 89; (b) p 97.
(7) Iyer, V. N.; Szybalski, W. *Science* **1964**, *145*, 55.
(8) Szybalski, W.; Iyer, V. N. *Microb. Genet. Bull.* **1964**, *21*, 16.
(9) Iyer, V. N.; Szybalski, W. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *0*, 355.
(10) Matsumoto, I.; Lark, K. G. *Exp. Cell Res.* **1963**, *32*, 192.
(11) Weissbach, A.; Lisio, A. *Biochemistry* **1965**, *4*, 196.
(12) Weiss, M. J.; Redin; G. S.; Allen, G. R., Jr.; Dornbush, A. C.; Lindsay, H. S.; Poletto, J. F.; Remers, W. A.; Roth, R. H.; Sloboda, A. E. *J. Med. Chem.* **1968**, *11*, 742.
(13) Mercado, C. M.; Tomasz, M. *Antimicrob. Agents Chemother.* **1972**, *1*, 73.
(14) Small, G.; Setlow, J. I.; Kooistra, J.; Shapanka, R. *J. Bacteriol.* **1976**, *125*, 643.
(15) Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110.
(16) Moore, H. W. *Science* **1977**, *197*, 527.
(17) Patrick, J. B.; Williams, R. P.; Meyer, W. E.; Fulmor, W.; Cosulich, D. B.; Broschard, R. W.; Webb, J. S. *J. Am. Chem. Soc.* **1964**, *86*, 1889.
(18) Hornemann, U.; Ho, Y.; Mackey, J. K., Jr.; Srivastawa, C. *J. Am. Chem. Soc.* **1976**, *98*, 7069.
(19) Tomasz, M.; Mercado, C. M.; Olson, J ; Chatterjie, N. *Biochemistry* **1974**, *13*, 4878.
(20) Webb, J. S.; Cosulich, D. B.; Mowat, J. H.; Patrick, J. B.; Broschard, R. W.; Meyer, W. E.; Williams, R. P.; Wolf, C. F.; Fulmor, W.; Pidacks, C.; Lancaster, J. E. *J. Am. Chem. Soc.* **1962**, *84*, 3185.
(21) Stevens, C. L.; Taylor, K. G.; Mink M. E.; Marshall, W. S.; Noll, K.; Shah, G. D.; Uzu, K. *J. Med. Chem.* **1965**, *8*, 1.
(22) Taylor, W. G.; Remers, W. A. *J. Med. Chem.* **1975**, *18*, 307.
(23) The pK_a' of the 2-amino groups of **2** and **3** is 6.5.[21] Thus **8a** is predicted to be zwitterionic at pH 3.5, negative at pH 7.2, and more negative at pH 10.0. **8b** is predicted to be zwitterionic at pH 3.5, very slightly negative at pH 7.2, more negative at pH 10.0.
(24) Ames, B. N.; Dubin, D. T. *J. Biol. Chem.* **1960**, *235*, 769.
(25) Seaman, E. *Methods Enzymol.* **1968**, *12B*, 218.
(26) Smith, J. D. In "The Nucleic Acids", Vol. I; Chargaff, E., Davidson, J. N., Ed.; Academic Press: New York, 1965; p 267.
(27) Windmueller, H. G.; Kaplan, N. O. *J. Biol. Chem.* **1961**, *236*, 2716.
(28) For a recent representative example, see Furuichi, Y.; Morgan, M.; Muthukrishnan, S.; Shatkin, A. J. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 362.
(29) This value was determined in 50% methanol–water.[21] Under our reaction conditions of relatively high ionic strength (0.34 M phosphate), a somewhat higher pK_a' is expected, consistent with the observed 4.0 value.
(30) Lown, J. W.; Weir, G. *Can. J. Biochem.* **1978**, *56*, 296.
(31) Taylor, W. G.; Remers, W. A. *Tetrahedron Lett.* **1974**, 3483.
(32) Cheng, L.; Remers, W. A. *J. Med. Chem.* **1977**, *20*, 767.
(33) Christensen H. N. *J. Biol Chem.* **1940**, *135*, 399.
(34) Davies, W.; Ross, W. C. J. *J. Chem. Soc.* **1952**, 4295.
(35) Price, C. C.; Gaucher, G. M.; Koneru, P.; Shibakawa, R.; Sowa, J. R.; Yamaguchi, M. *Biochim. Biophys. Acta* **1968**, *166*, 327.
(36) Brown, D. M.; Osborne, G. O. *J. Chem. Soc.* 1957, 2590.
(37) Sanno, Y.; Tanaka, K. *Chem. Pharm. Bull.* **1962**, *10*, 231.
(38) Dunn, D.; Hall, R. H. In "Handbook of Biochemistry and Molecular Biology. Nucleic Acids", Vol. I; Fasman, G. D., Ed.; CRC Press, Cleveland, Ohio, 1975; p 65.
(39) Gullino, P. M. *Adv. Exp. Med. Biol.* **1975**, *75*, 521.