72-4; ethyl *p*-aminobenzoate, 94-09-7; propargylbromide, 106-96-7; 2-butyn-1-ol, 764-01-2; methanesulfonyl chloride, 124-63-0; 2-butyn-1-ol mesylate, 61493-85-4; diethyl (*p*-aminobenzoyl)-L-glutamate, 13726-52-8; (bromomethyl)cyclopropane, 7051-34-5; *N*-*tert*-butoxycarbonyl-L-glutamic acid α-benzyl ester cesium salt, 64196-63-0; *N*-*tert*-butoxycarbonyl-L-glutamic acid α-benzyl ester, 30924-93-7; dihydrofolate reductase, 9002-03-3; thymidylate synthase, 9031-61-2.

Mitomycin C Analogues with Increased Metal Complexing Ability

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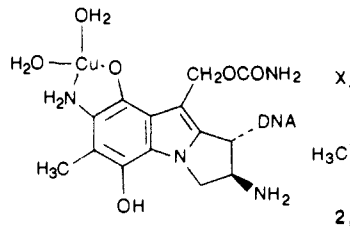
Twenty-three new mitomycin C analogues designed to have increased metal complexing ability were synthesized and tested against P388 leukemia in mice. Their ability to complex Cu(II) was revealed by the shifts in their UV absorption spectra caused by this metal. One analogue was clearly more active than mitomycin C in the antitumor assay and two others had good activity. Correlation between antitumor activity and Cu(II) complexing ability was ambiguous. The most active compounds were either not complexers or they were complexers limited to the 2-(2-pyridyl)alkyl type substituent on N7. A variety of amino acid substituents on N7 showed only weak antitumor activity.

One of the ways in which mitomycins produce cytotoxic effects is based on their ability to generate reactive species, including hydrogen peroxide and hydroxyl radicals, when they are reduced and reoxidized by air.[1,2] These species are produced even when the mitomycins are bound to DNA, and they cause single-strand cleavage of the DNA. Formation of the reactive species is promoted by metal ions such as Cu(II) and inhibited by catalase, superoxide dismutase, general radical scavengers, and sequestering agent.[1] These observations suggest processes similar to those involved in strand cleavage by bleomycin or streptonigrin.[3,4] It has been proposed that the interaction of mitomycin hydroquinone with molecular oxygen in the presence of metal ions results in the formation of mitomycin semiquinone radical and superoxide radical, which undergo further transformation into hydrogen peroxide and hydroxyl radical.[1] The semiquinone radical has been detected by its electron paramagnetic resonance spectrum.[5] No structure has been proposed for the metal complex thought to be formed from mitomycin hydroquinone, although 1 is an obvious possibility with copper(II).

Our interest in mitomycin metal complexes was stimulated by a recent report that Cu(II) ions strongly promoted the strand scission of bacteriophage 0X174 by mitomycin C (2) and sodium dithionite.[6] Based on the possibility that mitomycin–metal complexes might be taken up by tumor cells and show an enhanced cytotoxic effect, we attempted the preparation of complexes from a variety of mitomycins and metals such as Zn(II), Cu(II), and Pt(II).[7] Mitomycin C did not form stable complexes, but rearranged to a 2-aminomitosene that was capable of complex formation. Analogues, such as the N^7-[2-(2-pyridyl)ethyl] derivative of mitomycin C (4), that contained basic nitrogens formed stable metal complexes without rearrangement. Testing of the resulting metal complexes against P388 leukemia in mice showed some antitumor activity, but it was much less than that of mitomycin C. On the other hand, the uncomplexed N^7-[2-(2-pyridyl)ethyl] derivative (4) had enhanced antitumor activity. Thus result suggested that decreased activity of the metal complexes might be caused by poor cell uptake, whereas the mitomycin analogue 4 with good potential complexing ability might be taken up adequately and then acquire metal ions from constituents within the cell.[7]

The present study is based on the idea that mitomycin C analogues with enhanced metal complexing ability might show improved antitumor activity. It is an extension of the observation that 4 and certain other mitomycin derivatives, for example the N^7-[(dimethylamino)ethyl] derivative, were very active in the P388 assay.[7,8] Because it was not obvious at the start which functional groups would be most effective in improving antitumor activity, we chose a variety of different types: some based closely on 4, others with different heterocycles such as furan and imidazole, and still others with an array of aliphatic amines, ethers, and alcohols. One type of analogue receiving special attention was the amino acid substituent. Our previous preparation and testing of the glycine and



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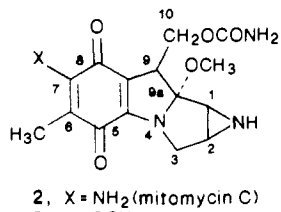
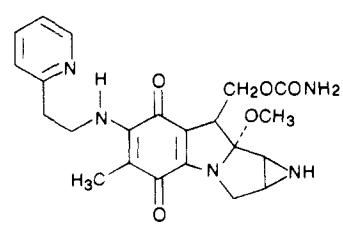


2, X = NH_2 (mitomycin C)
3, X = OCH_3 (mitomycin A)



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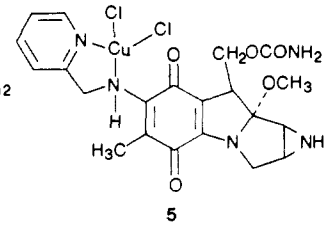

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‡Bristol-Myers Company.

(1) Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110.
(2) Tomasz, M. *Chem.-Biol. Interact.* **1976**, *13*, 89.
(3) Takita, T.; Muraoka, Y.; Nakatani, T.; Fujita, A.; Iitaka, Y; Umezawa, H. *J. Antibiot. (Tokyo)* **1978**, *31*, 1073.
(4) Cone, R.; Hasan, S. K.; Lown, J. W.; Morgan, A. R. *Can. J. Chem.* **1976**, *54*, 219.
(5) Pan, S.-S.; Andrews, P. A.; Glover, C. J.; Bachur, N. *J. Biol. Chem.* **1984**, *259*, 959.
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(7) Iyengar, B. S.; Takahashi, T.; Remers, W. A.; Bradner, W. T. *J. Med. Chem.* **1986**, *29*, 144.
(8) Iyengar, B. S.; Sami, S. M.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1983**, *26*, 16.

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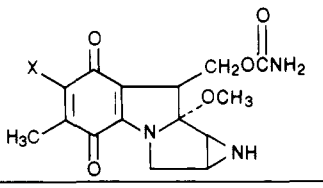
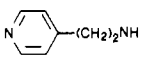
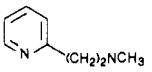
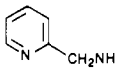
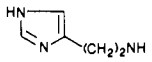
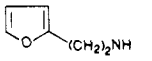
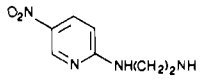
Table I. Preparation and Properties of New Mitomycin C Analogues[a]



no.	X	method	yield, %	solvents for recrystn or chromatog	solvent impurity	mp, °C (dec)	^{1}H NMR signals for the 7-substituent;[b] signals for the solvent impurity
6		B	74	CH_3OH–CH_2Cl_2	$0.4CH_2Cl_2$	>200	2.9–3.2 (m, 2), 3.8–4.2 (m, 2), 7.3 (m, 2), 7.8 (br s, 1), 8.5 (br s, 1)[c]; 5.33 (s)
7		B	39	CH_3OH–CH_2Cl_2	$1.5CH_3OH$	82–85	2.10 (s, 3), 3.3–4.3 (m, 4), 6.6–8.0 (m, 3), 8.2–8.8 (br s, 1), 3.3–3.4 (br s)
8[g]		A	49	CH_3OH–CH_2Cl_2	$1CH_3OH$	83–87	3.98 (s, 2), 5.3 (br s, 1), 7.0–7.9 (m, 3), 8.57 (d, 1); 3.3–3.4 (br s)
9[h]		B	71	CH_3OH–CH_2Cl_2	$4H_2O$	indef	2.5–3.0 (m, 4), 6.8 (s, 1), 7.55 (s, 1)[d]
10[i]		B	61	CH_3OH–CH_2Cl_2		88–92	2.47–3.03 (m, 4), 5.83–6.60 (m, 3), 7.27 (d, 1)
11[j]	$H_2N(CH_2)_3NH$	A	50	CH_2Cl_2–hexane	0.5hexane	>200	1.26 (br s, 2), 2.50–3.00 (m, 6), 5.70 (br s, 1)
12[k]	$HO(CH_2)_2O(CH_2)_2NH$	A	42	CH_2Cl_2–hexane	$1CH_3OH$	99–102	3.39–3.93 (br s, 9), 6.43–6.80 (br s, 1); 3.3–3.4 (br s)
13	$HO(CH_2)_2NH(CH_2)_2NH$	A	58	CH_2Cl_2–hexane	0.5hexane	115–118	2.70 (s, 7), 3.73 (br s, 3); 0.9–1.1 (m)
14	$H_2N(CH_2)_2NH(CH_2)_2NH$	A	25	acetone	1acetone	79–83	1.1 (br s, 3), 2.2–2.95 (br s, 4), 3.2–3.8 (m, 4), 7.0–7.3 (br s, 1); 2.03 (s)
15[l]	$(H_2NCH_2CH_2)_2N$	B	43	C_2H_5OH	$1H_2O$	>250	1.3 (t, 4), 2.65 (br s, 8)
16	$H_2NC(=NH)NH(CH_2)_4NH$	B	70	CH_3OH–CH_2Cl_2	$3CH_3OH$	95–99	1.6–1.9 (br s, 4), 2.6–3.4 (br s, 4)[e]; 3.3–3.4 (br s)
17	$H_2NCH_2CH(OH)CH_2NH$	B	90	CH_3OH–ether	$1.5H_2O$	>300	1.0–1.3 (br s, 2), 2.7 (m, 2), 3.2–4.2 (m, 4); 7.5 (br s)[f]
18[m]	$HOCH_2CH(OH)CH_2NH$	B	72	CH_3OH–ether	$0.5H_2O$	>300	3.5–3.77 (m, 5), 4.1–4.3 (m, 2)[f]
19[n]	$(HOCH_2CH_2)_2N(CH_2)_3NH$	B	55	CH_3OH–CH_2Cl_2	$1CH_3OH$	81–85	2.2–2.90 (m, 8), 3.33 (s, 2), 3.4–3.9 (m, 6), 7.91 (t, 1); 3.3–3.4 (br s)
20[o]		A	56	acetone–CH_2Cl_2	1acetone	76–79	3.30–4.0 (m, 4), 6.17–6.67 (br s, 2), 8.1 (d, 1), 8.20 (d, 1), 9.03 (s, 1); 2.03 (s)
21[p]	L-$CH_3CH(CO_2H)NH$	C	52	CH_3OH–CH_2Cl_2	$1CH_2Cl_2$	211–221	1.5 (d, 3), 3.76 (q, 1)[e]; 5.33 ns)
22	D-$CH_3CH(CO_2H)NH$	C	43	CH_3OH–CH_2Cl_2	$1CH_2Cl_2$	220–230	1.5 (d, 3), 3.80 (q, 1)[e]; 5.33 (s)
23[q]	$HO_2C(CH_2)_2NH$	C	43	CH_3OH	$2.5CH_3OH$	98–102	2.5 (br s, 2), 3.3 (br s, 2)[e]; 3.3–3.4 (br s)
24[r]	L-$H_2NCH(CO_2H)(CH_2)_2NH$	B	16	CH_3OH		>250	3.2–4.3 (br s, 7), 7.7–8.2 (br s, 1)[c]
25	L-$HOCH_2CH(CO_2C_2H_5)NH$	B	23	CH_3OH–CH_2Cl_2	$2CH_3OH$	200–205	1.3 (t, 3), 3.6–4.5 (br s, 5)[d]; 3.3–3.4 (br s)
26[s]	L-$HOCH_2CH(CO_2H)NH$	C	34	CH_3OH	$2CH_3OH{\cdot}0.5H_2O$	>190	3.8–4.1 (br s, 3)[e]; 3.3–3.4 (br s)
27	L-$CH_3S(CH_2)_2CH(CO_2CH_3)NH$	B	30	acetone–pentane	1acetone	83–85	1.60–2.40 (m, 3), 2.10 (s, 3), 2.43–3.0 (m, 2), 3.80 (s, 3), 8.3–9.3 (br s, 1)[c]; 2.04 (s)
28[t]	L-$H_2NC(=NH)NH(CH_2)_2C(CO_2H)HNH$	C	71	CH_3OH	$1H_2O$	155–158	1.6–1.9 (br s, 4), 2.6–3.4 (br s, 4)[e]

[a] Analytical results were within ±0.40% of theoretical values for all elements (C, H, N), except as shown in subsequent footnotes. In examples, the solvent impurities indicated in the table had to be added to reconcile the calculated and found values for these elements. NMR signals for the solvent impurities are given in the table. It was not possible to obtain exact ratios for protons in the solvent impurities with respect to those in the compound by integrating the spectra. [b] The solvent for NMR was $CDCl_3$ unless specified otherwise. The following solvent specifications are used: [c] Me_2SO-d_6; [d] $CDCl_3$ + CD_3OD; [e] D_2O; [f] $CDCl_3$ + Me_2SO-d_6. [g] H: calcd, 5.95; found, 5.25. N: calcd, 15.30; found, 14.74. [h] H: calcd, 6.40; found, 5.53. N: calcd, 16.80; found, 15.86. [i] N: calcd, 12.30; found, 10.94. [j] C: calcd, 58.05; found, 57.34. [k] C: calcd, 52.86; found, 52.31. N: calcd, 12.32; found, 11.61. [l] N: calcd, 19.17; found, 20.12. [m] H: calcd, 6.45; found, 5.87. N: calcd, 6.63; found, 16.13; found, 15.54. [n] N: calcd, 13.69; found, 12.86. [o] C: calcd, 53.86; found, 53.34. [p] C: calcd, 46.45; found, 46.99. [q] H: calcd, 6.63; found, 5.90. [r] H: calcd, 5.79; found, 6.35. N: calcd, 16.08; found, 12.46. [s] H: calcd, 6.31; found, 5.77. [t] H: calcd, 6.13; found, 6.56.

glycine methyl ester analogues of mitomycin C revealed potent antitumor activity and suggested further exploration of this type.[9] A major concern we had was that if a compound was too strong in complexing metal ions it might immediately capture them and not be able to penetrate cells. This appeared to be the case with our previously prepared N^7-[2-(1-piperizinyl)ethyl] analogue.[7] In the absence of a clear idea where the optimum complexing ability might lie, we relied on the number and variety of analogues to determine this point empirically.

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Results and Discussion

The 23 new compounds prepared in this investigation

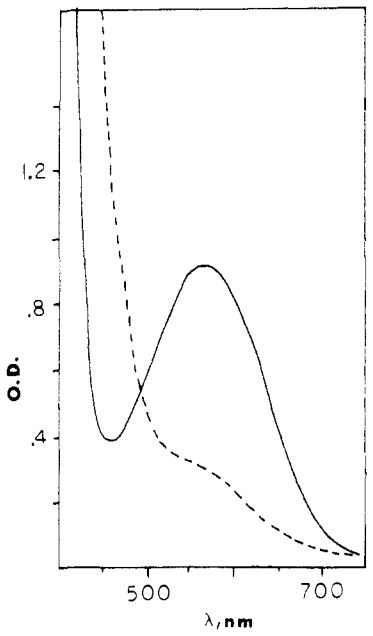


Figure 1. Visible absorption spectrum of 14 (solid line) and its rearrangement product 15 (dashed line) in methanol.

are listed in Table I. They were made by the same general method used for previous mitomycin C analogues, treatment of mitomycin A with the appropriate primary or secondary amine.[7-9] The solvent and added base (if any) were chosen to suite the particular amine. Thus, reactive primary amines were used in methylene chloride (method A). Other amines were used in methanol, with sodium methoxide or triethylamine added if the amine was obtained as a salt (method B). For the amino acids, a water–triethylamine mixture was the solvent (method C). The yields, melting points, and NMR data for the new substituents are given in Table I. There was one reaction that took an unexpected course. When mitomycin A (**3**) was treated with diethylenetriamine in methylene chloride, the product **14** resulted from preferential reaction with a primary amine. However, in methanol the product **15** resulted from reaction with the secondary amine. It was possible to convert **14** into **15** by dissolving it in methanol. Within 24 h the color changed from blue to green (Figure 1), which is characteristic of the difference between primary and secondary amine substituents at C7 of mitomycins. This is the first example we have observed of the displacement of one amino group by another at the C7 position, and this is despite many attempts to react mitomycin C directly with other amines. Probably the intramolecularity of the reaction facilitates this displacement. There was no change in **14** when it was kept in chloroform for 24 h.

In order to gain insight into the potential metal complexing ability of our mitomycin analogues, were made two different studies. One involved the ability of compound **4** to complex various divalent metal ions in absolute methanol, as indicated by shifts in its ultraviolet absorption maximum at 367 nm (π–π^* transition). As shown in structure **5**, complex formation probably involves the 7-amino nitrogen, which is part of the mitomycin ultraviolet chromophore. Thus, complexation should perturb the chromophore. Table II shows that Ni(II) and Cu(II) clearly give the greatest shifts, Co(II) and Zn(II) give small shifts, and the other metal ions in the list had no effect. Figure 2 shows the spectrum of **4** before and after complexation with Cu(II). The second study measured the relative shifts in the 367-nm peak produced by Cu(II) for various mitomycin analogues. Methanol was used because some of the compounds were not soluble in water. Results of this study are given in Table III. They show that the analogues having a basic nitrogen atom two or three atoms removed from N7 cause significant shifts in λ_{max} (9–22 nm), whereas those with an oxygen atom in the corresponding site have no effect on wavelength. The analogue **7**, which is related to **4** but has a tertiary 7-amine, shows a smaller shift than **4**, whereas **8**, also related to **4** but with a shorter chain, shows a larger shift. The latter result indicates the difference between a five-membered chelate (**5**) and a six-membered chelate. One of the most significant results in Table III is that the 2-(4-pyridyl)ethyl analogue **6** of **4** shows no λ_{max} shift in the presence of Cu(II). It supports the idea that copper complexation involves a chelate formed from N7 and a second nitrogen two or three carbon atoms removed. Analogue **6** cannot form such a chelate. Among the analogues without a pyridine ring, **13**, **14** and **19** showed the greatest shifts on treatment with Cu(II).

Table II. Ability of Various Metal Ions To Bind Compound 4[a]

ion	λ_{max}, nm	$\Delta\lambda_{max}$	ion	λ_{max}, nm	$\Delta\lambda_{max}$
Mg(II)	366.7	0	Cu(II)	382.6	15.9
Ca(II)	366.7	0	Zn(II)	368.4	1.7
Mn(II)	366.7	0	Cd(II)	366.7	0
Co(II)	370.0	3.3	Hg(II)	366.7	0
Ni(II)	385.0	18.3	none	366.7	0

[a] A solution of 4 in absolute methanol at 8×10^{-5} M was treated with 1 equiv of anhydrous metal chloride at 27 °C.

Table III. Copper Complexation and Partition Coefficients of the Mitomycin Analogues

compd	$\Delta\lambda_{max}$,[a] nm	log P[b]	compd	$\Delta\lambda_{max}$,[a] nm	log P[b]
4	15.9	−0.09	**15**	9.2	
6	0	0.31	**16**	0	
7	6.7	0.37	**19**	19.2	−0.36
8	21.7	−0.09	**20**	0	0.77
9	10.0	−0.19	**23**	9.2	
10	0		**24**	10.0	
11	11.7	−1.30	**25**	11.7	
12	0	−0.71	**26**	7.5	
13	18.3	−0.57	**28**	10.0	
14	15.8	−1.14			

[a] Bathocromic shift of the λ_{max} at 367 nm. The compound and $CuCl_2$ are both 8×10^{-5} M in absolute methanol, and the blank is 8×10^{-5} M in $CuCl_2$. [b] Distribution between *n*-octanol and water containing phosphate buffer at pH 7.4. Determination by the method of Hansch, C.; Muir, R. M.; Fujita, T.; Malongy, P. P.; Geiger, F.; Struch, M. J. *J. Am. Chem. Soc.* **1963**, *85*, 2817.

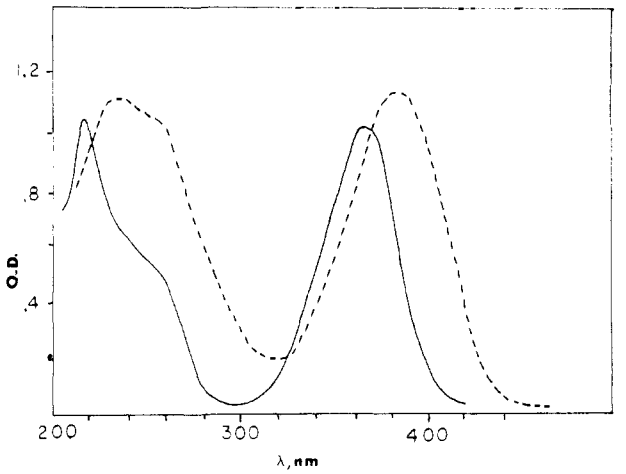


Figure 2. Ultraviolet absorption spectrum of 4 in methanol before (solid line) and after (dashed line) addition of cupric chloride. Both components were 8×10^{-5} M.

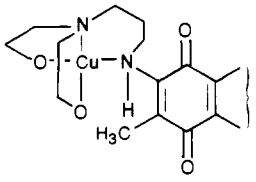
Table IV. Complex Formation Between **4** and **23** with Various Ratios of $CuCl_2$ in Water

compd	ratio compd/$CuCl_2$	$\Delta\lambda_{max}$,[a] nm
4	4:1	1.5
	2:1	5.0
	1:1	9.0
	1:2	10.0
	1:4	10.0
23	4:1	2.5
	2:1	6.5
	1:1	9.5
	1:2	9.5
	1:4	9.5

[a] Bathochromic shift of the λ_{max} at 367nm in water at pH 7.0. The mitomycin analogue is at 8×10^{-5} M, and the $CuCl_2$ is in the indicated multiples of this concentration. The blank contained $CuCl_2$ at the same concentration as in the sample.

The compounds are capable of forming tridentate or tetradentate chelates. For example, structure **29** might be formed from **19**. The magnitudes of shifts for the compounds in Table III do not necessarily imply relative binding strengths.

Amino acid derivatives **23–26** and **28** all showed significant ultraviolet absorption maximum shifts when treated with Cu(II). The other amino acid derivatives were not measured. It is possible to draw satisfactory chelate structures for each of the expected interactions. One is illustrated (**30**) for the serine ethyl ester analogue **25**, which showed the greatest shift.



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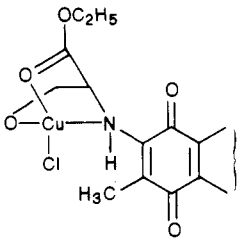

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In the two studies described above, methanol was the solvent and fixed 1:1 ratios of the metal ion and mitomycin were used. Because studies in methanol cannot be extrapolated to biological systems, we made an ultraviolet absorption study with cuprous chloride in water. A range of ratios of mitomycin analogue to metal ion (4:1, 2:1, 1:1, 1:2, 1:4) were examined to confirm the stoichiometry of complex formation. Rather than examine every analogue, we chose two that were representative of compounds which gave significant effects in methanol. Compound **4** represents the analogues with a basic nitrogen atom in the 7-substituent, and compound **23** represents those with a carboxylic acid in this substituent (amino acid analogues). The results, given in Table IV, show that the bathocromic shift in λ_{max} increases with increasing amounts of cuprous chloride until the 1:1 ratio is obtained, but does not change beyond that ratio. They suggest that 1:1 complexes are formed with relatively good affinity. This suggestion is consistent with our ability to isolate stable 1:1 complexes from **4** and another mitomycin analogue in a previous study.[7]

Octanol–water partition coefficients were determined on some of the analogues. They are listed in Table III. Partition coefficients might be important to antitumor activity because they influence the ability of compounds to enter tumor cells. In our previous experience, one series of mitomycin analogues, the N^7-phenyl derivatives,[10] gave an excellent correlation between antitumor potency and log *P*. However, in two other series there was so much scatter in the data that the correlation was not statistically significant.,[7,8]

Table V. Antitumor Activity of Mitomycin C Analogues[a]

	P-388 leukemia in mice				
	max effect, % T/C		OD,[c]	MED,	TR,
compd	compd	mit C[b]	mg/kg	mg/kg	OD/MED
4	>375 [4]	319	25.6	0.2	128
6	167	233	25.6	3.2	8
7	300 [2]	319	12.8	<0.2	>64
8	233 [1]	272	25.6	1.6	16
9	163	213	25.6	1.6	16
10	328 [2]	156	25.6	1.6	16
11	147	279	25.6	6.4	4
12	181	213	25.6	0.4	64
13	133	156	12.8	12.8	1
14	100	178			
15	129	276	25.6	3.2	8
16	156	263	25.6	1.6	16
17	122	>344	12.8	12.8	
18	175	205	25.6	6.4	4
19	137	279	25.6	25.6	1
20	144	250	12.8	2.6	8
21	105	132	12.8		
22	109	132	12.8		
23	133	194	25.6	25.6	1
24	139	206	25.6	6.4	4
25	156	194	25.6	3.2	8
26	138	263	25.6	6.4	4
27	131	250	6.4	3.2	2
28	138	263	25.6	6.4	4
mit C	132–>344		3.2–4.8	0.2	16–24

[c] Determined at Bristol-Myers Co., Syracuse, NY. A tumor inoculum of 10^6 ascites cells was implanted ip in CDF_1 female mice. Six mice were used at each dose of the mitosane, and 10 control mice were injected with saline. A control group of six mice at each dose reveived mitomycin C in the same expteriment: MST = median survival time; max effect (% T/C) = MST treated/MST control × 100 at the optimal dose (OD); MED = minimum effective dose (% T/C 125); TR = therapeutic ratio (OD/MED). The number of 30-day survivors at the optimal dose is given in brackets beside the maximum effect. [b] Mitomycin C. [c] Optimal dose.

Biological Activity. All of the compounds prepared in this investigation were tested against P388 lymphocytic leukemia in mice. Their activities are compared with those of mitomycin C and analogue **4** in Table V. The compounds were not all tested at the same time, but each test group contained a mitomycin C positive control. Consequently, compounds should not be compared directly with each other, but compared according to how each one relates to its mitomycin C control. The control value for each analogue (% T/C) is given in the third column of Table V. Among the compounds listed in Table V, **4** and one other, the (furylethyl)amino analogue **10**, are clearly more active than mitomycin C. It is difficult to say which of the two is more effective because the controls vary so widely, but **10** certainly is much more active than its control. Compounds **7** and **8**, both analogues of **4**, are nearly as active as mitomycin C and they give 30-day survivors. The low minimum effective dose (MED) of **7** is typical of secondary amines, which are easier to reduce (bioactivate) than primary animes because steric hindrance limits conjugation of the nitrogen with the quinone.[11] Other compounds showing moderately good activity are **12** and **18**, both of which have no nitrogen atoms in the substituent on N7. The remainder of the analogues have low or no activity against P388 leukemia. One of the most inter-

(10) Sami, S. M.; Iyengar, B. S.; Tarnow, S. E.; Remers, W. A.; Bradner, W. T.; Schurig, J. E. *J. Med. Chem.* **1984**, *27*, 701.

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esting comparisons in Table V is between **4** and its 4-pyridyl isomer **6**. The latter is much less active than the former or the other 2-(2-pyridyl)alkyl analogues **7** and **8**. Although the glycine analogue of mitomycin C had good antitumor activity, none of the new amino acid analogues **21–28** in Table V showed useful activity. However, the best of these compounds, serine ethyl analogue **25**, gives the largest $\Delta\lambda_{max}$ with Cu(II) ion.

Conclusions

Comparison of the antitumor activities of compounds in Table V with their Cu(II) complexing ability in Table III gives a result that difficult to rationalize. The two types of compounds with good antitumor activity are the 2-pyridyl derivatives, which complex Cu(II), and the compounds such as **10** and **12**, which do not complex Cu(II). Other good complexing agents such as **14**, **15**, and **19** are virtually inactive. This raises the question as to whether there is some unique feature to the complexing ability of 2-pyridyl derivatives, perhaps an optimal association constant, that gives them an advantage over other complexing agents. The pyridine nitrogen is considerably less basic (pK_a = 5.2) than aliphatic amines (pK_a = 10). It could be that both the 2-pyridyl derivatives and the noncomplexing agents penetrate tumor cells, whereas the analogues with aliphatic amines do not. If this were the case, it would be difficult to explain the substantial difference in activity between **4** and its 4-pyridyl isomer **6**. Possibly this difference is based on specific hydrogen-bond formation to DNA. Although none of the amino acid derivatives had good activity, there was a hint that complexing might be of some benefit, as shown by serine ethyl ester derivative **25**.

The partition coefficients in Table III are not useful in correlating antitumor activity. All of the analogues are relatively hydrophilic, and there is no statistical significance to an attempted correlation with MED. The wide variation in structural types (primary amines, secondary amines, heterocyclic rings) might be partly responsible for the lack of correlation.

Thus, there is no simple way in which to account for the results of this investigation. The preparation of additional metal complexing mitomycin analogues is not compelling, but further work related to N7-substitutes bearing heteroaryl rings, as exemplified by **4** and **10**, ought to be valuable.

Experimental Section

General. Melting points were determined on a Laboratory Instruments Mel-Temp apparatus and are uncorrected. UV and visible spectra were taken on a Beckman DU-8 spectrophotometer. ^1H NMR spectra were recorded on a Varian EM-360 (60 MHz) spectrometer or a JEOL FX90Q (90 MHz) spectrometer with tetramethylsilane as the internal standard. Elemental analyses were performed by the Analytical Center, University of Arizona or Mic Anal, Tucson, AZ.

Methods for the Preparation of Mitomycin C Analogues. Method A. A solution of mitomycin A (**3**, 100 mg, 0.29 mmol) and the appropriate amine (0.6–1.0 mmol) in 20 mL of methylene chloride was stirred under N_2 at room temperature until the conversion was complete according to TLC (5–24 h). The solution was concentrated under reduced pressure, and the residue was purified by preparative TLC on precoated silica gel plates (20 × 20 × 0.2 cm) with CH_3OH–$CHCl_3$ (1:4) as the developing solvent. The products were recrystallized from a CH_3OH–CH_2Cl_2 mixture with cooling. Yields and physical properties are given in Table I.

Method B. A solution of mitomycin A (100 mg, 0.29 mmol) in either 8 mL of methanol or 5 mL of *N,N*-dimethylformamide was treated with the appropriate amine or its salt (0.6 mmol). If the salt was used, 0.6 mmol of either sodium methoxide or triethylamine was added to the solution. When the conversion was complete according to TLC (15 min to 10 h), the solvent was removed under reduced pressure and the residue was purified by chromatography as described in method A.

Method C. Mitomycin A (100 mg, 0.29 mmol) was added to a solution of the amino acid (0.29 mmol) in 5 mL of water and 5 mL of triethylamine. The resulting solution was stirred at room temperature for 1 h, during which time the reaction went to completion according to TLC. Solvents were removed under reduced pressure, and the residue was purified by preparative TLC on silica gel plates (20 × 20 × 0.2 cm) with CH_3OH–$CHCl_3$ (3:7) as the developing solvent.

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Registry No. **2**, 50-07-7; **3**, 4055-39-4; **6**, 103094-99-1; **7**, 103131-66-4; **8**, 103095-00-7; **9**, 93073-93-9; **10**, 103095-01-8; **11**, 103095-02-9; **12**, 93073-92-8; **13**, 93073-91-7; **14**, 103095-03-0; **15**, 103095-04-1; **16**, 103095-05-2; **17**, 103095-06-3; **18**, 103095-07-4; **19**, 103095-08-5; **20**, 93073-97-3; **21**, 103095-09-6; **22**, 103188-99-4; **23**, 103095-10-9; **24**, 103095-11-0; **25**, 103095-12-1; **26**, 103095-13-2; **27**, 84397-33-1; **28**, 103095-14-3; $H_2N(CH_2)_3NH_2$, 109-76-2; $HO(CH_2)_2O(CH_2)_2NH_2$, 929-06-6; $HO(CH_2)_2NH(CH_2)_2NH_2$, 111-41-1; $H_2N(CH_2)_2NH(CH_2)_2NH_2$, 111-40-0; $H_2NC(=NH)NH(CH_2)_4NH_2$, 306-60-5; $H_2NCH_2CH(OH)CH_2NH_2$, 616-29-5; $HOCH_2CH(OH)CH_2NH_2$, 616-30-8; $(HOCH_2CH_2)_2N(CH_2)_3NH_2$, 4985-85-7; (L)-$CH_3CH(CO_2H)NH_2$, 56-41-7; (D)-$CH_3CH(CO_2H)NH_2$, 338-69-2; $HO_2C(CH_2)_2NH_2$, 107-95-9; (L)-$H_2NCH(CO_2H)(CH_2)_2NH_2$, 1758-80-1; (L)-$HOCH_2CH(CO_2C_2H_5)NH_2$, 2788-84-3; (L)-$HOCH_2CH(CO_2H)NH_2$, 56-45-1; (L)-$CH_3S(CH_2)_2CH(CO_2CH_3)NH_2$, 10332-17-9; (L)-$H_2NC(=NH)NH(CH_2)_2CH(CO_2H)NH_2$, 14191-90-3; Co(II), 7440-48-4; Ni(II), 7440-02-0; Cu(II), 7440-50-8; Zn(II), 7440-66-6; 4-(2-aminoethyl)pyridine, 13258-63-4; 2-(2-methylaminoethyl)pyridine, 5638-76-6; 2-aminomethylpyridine, 3731-51-9; 4-(2-aminoethyl)imidazole, 51-45-6; 2-(2-aminoethyl)furan, 1121-46-6; 2-(2-aminoethylamino)-5-nitropyridine, 29602-39-9.