6-(Diacetoxymethyl)pyridine-2-carbothioamide (10). Compound **9** (6 g, 25 mmol) was dissolved in a solution of 5.5 mL of $(C_2H_5)_3N$ in 50 mL of anhydrous pyridine. Hydrogen sulfide gas was bubbled through the solution for 1 h. The solution was then diluted with H_2O (200 mL) and extracted several times with EtOAc. The EtOAc extracts were dried (Na_2SO_4) and evaporated under vacuum to leave a residue which was chromatographed on a silica gel column, eluting with a mixture C_6H_6–EtOAc (4:1). Evaporation of the fractions containing **10** gave 5.42 g (79%) of a solid which was recrystallized from a mixture of EtOH and H_2O, mp 100–102 °C. Anal. ($C_{11}H_{12}N_2O_4S$) C, H, N, S.

6-Formylpyridine-2-carbothioamide (11). A solution of **10** (0.9 g, 3.35 mmol) in 38 mL of a mixture EtOH–H_2O (1:1) containing 0.6 mL of concentrated HCl was refluxed for 30 min. The solution was neutralized with 2 N Na_2CO_3 and extracted several times with EtOAc. Evaporation of the solvent afforded a residue which was chromatographed on a short silica gel column, eluting with EtOAc. Evaporation of the fractions containing **11** gave 0.260 g (46.4%) of a solid, mp 147–149 °C. Anal. ($C_7H_6N_2OS$) C, H, N, S.

6-Formylpyridine-2-carbothioamide Oxime (3a). 6-(Diacetoxymethyl)pyridine-2-carbothioamide (1 g, 3.72 mmol) and NH_2OH·HCl (0.5 g, 7.19 mmol) in 50 mL of ethanol were refluxed for 30 min. The cold solution was neutralized with $NaHCO_3$ to pH 5, diluted with H_2O, and extracted several times with $CHCl_3$. Evaporation of the extracts left a residue which was chromatographed on a silica gel column, eluting with a mixture C_6H_6–EtOAc (9:1). Evaporation of the first eluate yielded **3a** (0.51 g, 76%), which was recyrstallized from ethanol, mp 185–186 °C. Anal. ($C_7H_7N_3OS$) C, H, N, S.

6-Formylpyridine-2-carbothioamide Semicarbazone (3b). **10** (1 g, 3.72 mmol) and semicarbazide (0.84 g, 11.1 mmol) in 50 mL of ethanol were refluxed for 5 h. A solid precipitated, which was filtered and recrystallized from ethylene glycol monomethyl ether to give 0.66 g (70%) of **3b**, mp 245–247 °C. Anal. ($C_8H_9N_5OS$) C, H, N, S.

6-Formylpyridine-2-carbothioamide Thiosemicarbazone (3c). **10** (1 g, 3.72 mmol) and thiosemicarbazide (0.48 g, 5.2 mmol) in 50 mL of ethanol and 0.5 mL of concentrated HCl were refluxed for 5 h. A solid precipitated, which was filtered and recrystallized from ethylene glycol monomethyl ether to yield 0.71 g (70%) of **3c**, mp 260–262 °C. Anal. ($C_8H_9N_5S_2$) C, H, N, S.

6-Formylpyridine-2-carbothioamide Phenylhydrazone (3d). **10** (1 g, 3.72 mmol) and phenylhydrazine hydrochloride (1.1 g, 8.8 mmol) in 50 mL of ethanol were refluxed for 8 h. The solution was evaporated to half volume and neutralized to pH 6 with 2 N $NaHCO_3$. A solid precipitated, which was filtered and recrystallized from ethanol to afford 0.71 g (65%) of **3d**, mp 193–194 °C. Anal. ($C_{13}H_{12}N_4S$) C, H, N, S.

6-Formylpyridine-2-carbothioamide *N*-Phenylimine (3e). **10** (1.1 g, 4.1 mmol) and aniline (2.5 g, 26.8 mmol) in 100 mL of anhydrous ethanol were refluxed for 2 h. Evaporation of the solvent gave a residue which was chromatographed on a silica gel column, eluting with C_6H_6–EtOAc (9.5:5). Evaporation of the eluates containing the first fraction yielded an oil, which under vacuum changed into a solid. The solid was washed with Et_2O, filtered, and dried under vacuum to give 0.3 g (30.3%) of **3e**, mp 136–138 °C. Anal. ($C_{13}H_{11}N_3S$) C, H, N, S.

Glyoxal 2-Phenylhydrazone Thiosemicarbazone (4a). Glyoxal 2-phenylhydrazone (1 g, 6.09 mmol) and thiosemicarbazide (0.61 g, 6.69 mmol) in 50 mL of ethanol were refluxed for 2 h. Evaporation of the solvent left a residue which was chromatographed on a silica gel column, eluting with C_6H_6–EtOAc (8:2). Evaporation of the fractions containing **4a** gave 1 g (66.9%) of product, mp 111–113 °C (from EtOAc–C_6H_6). Anal. ($C_9H_{11}N_5S$) C, H, N, S.

Dimethylglyoxal 2-Phenylhydrazone Thiosemicarbazone (4b). Dimethylglyoxal 2-thiosemicarbazone (1 g, 6.1 mmol) and phenylhydrazine (0.73 g, 6.8 mmol) in 50 mL of ethanol were refluxed for 1 h. After the solution was cooled, a solid precipitated, which was filtered and recrystallized from ethanol to yield 1 g (63.8%) of **4b**, mp 220–221 °C. Anal. ($C_{11}H_{15}N_5S$) C, H, N, S.

Bis(2,2′-bipyridyl-6-carbothioamide)iron(II) Sulfate (12). To 1 g (4.6 mmol) of **1a** dissolved in 30 mL of hot ethanol were added 0.645 g (2.3 mmol) of $FeSO_4·7H_2O$ dissolved in 2 mL of water. The addition product immediately precipitated as a dark-blue solid, which was filtered, washed with ethanol, and dried under vacuum: yield 66%; UV λ_{max} (methanol) 265 nm (log ϵ 4.30), 275 (4.71), 313 (4.34), 588 (3.65). Anal. ($C_{22}H_{16}FeN_6S_2·H_2SO_4$) C, H, N, S.

Bis(2,5′-bipyridyl-6-carbothioamide)iron(II) (13). A solution of 1 g of **12** in 10 mL of 0.5 N NaOH was shaken for 10 min. A dark blue solid precipitated, filtered, washed several times with water, and dried under vacuum: yield 99%; UV λ_{max} (methanol) 259 nm (log ϵ 4.50), 308 (4.45), 588 (4.14). This compound is paramagnetic. Anal. ($C_{22}H_{16}FeN_6S_2$) C, H, N, S.

Bis(6-formylpyridine-2-carbothioamide *N*-phenylimine)iron(II) Sulfate (14). To 0.7 g (2.9 mmol) of **3e** dissolved in 40 mL of ethanol was added 0.404 g (1.45 mmol) of $FeSO_4·7H_2O$ in 4 mL of water. The solution was shaken for 10 min and then was evaporated under vacuum. The dark-blue solid obtained was extracted several times with ethyl acetate, and then the product was filtered and dried under vacuum: yield 65%; UV λ_{max} (methanol) 228 nm (log ϵ 4.58), 317 (4.37), 634 (3.69). Anal. ($C_{26}H_{22}FeN_6S_2·H_2SO_4$) C, H, N, S.

Bis(6-formylpyridine-2-carbothioamide *N*-phenylimine)iron(II) (15). A solution of 0.24 g of **14** in 4 mL of 0.5 N NaOH was shaken for 10 min. A dark blue solid precipitated, filtered, and washed with water: yield 98%; UV λ_{max} (methanol) 226 nm (log ϵ 4.60), 318 (4.32), 637 (3.72). Anal. ($C_{26}H_{20}FeN_6S_2$) C, H, N, S.

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Synthesis and Antineoplastic Activity of Mitosene Analogues of the Mitomycins[1a]

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A series of 1-substituted mitosene analogues of the mitomycin antitumor antibiotics was prepared by total synthesis and screened for activity against P388 leukemia in mice. In general, analogues with moderately good leaving groups (mostly esters) at the 1 position were active, whereas analogues without such substituents were inactive or barely active. These results lend support to the idea that mitosenes with leaving groups at position 1 are capable of bifunctional alkylation of DNA in a manner similar to that of mitomycin C. The most active mitosenes were equal in potency (minimum effective dose) to a corresponding aziridinomitosene, but they were less effective in prolonging life span.

Mitomycin C (1) has been shown to cross-link double-helical DNA after reduction to the corresponding hydroquinones **2** (Scheme I).[2] This process, known as bioreductive alkylation,[3] is thought to be the main lethal event

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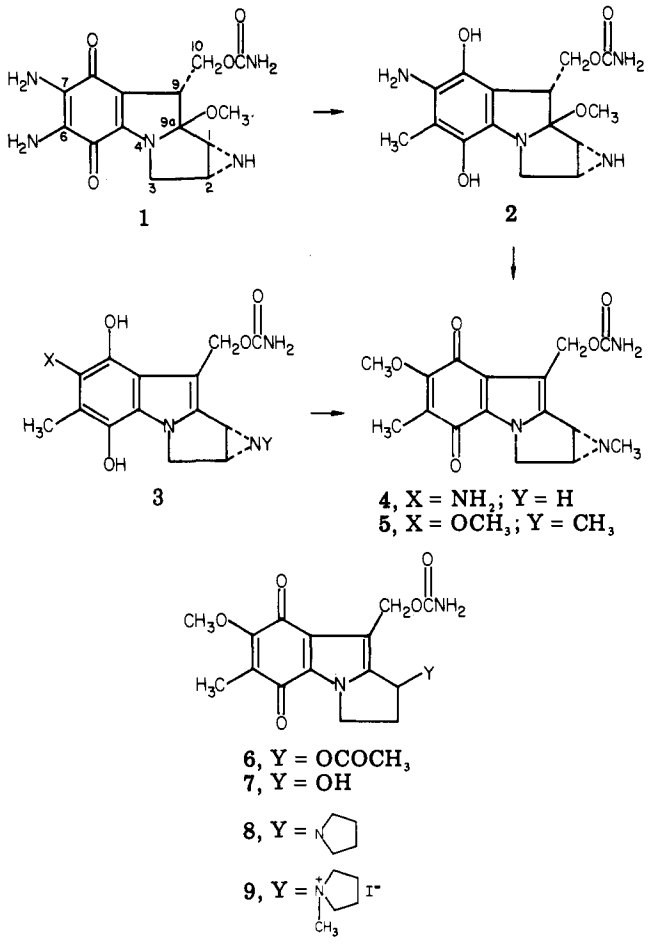
Scheme I



to tumor cells, although the generation of hydrogen peroxide by successive redox cycles of the DNA-bound mitomycin also is considered important.[4,5] Positions 1 and 10 appear to be the alkylating sites of mitomycin C, with their alkylating ability enhanced when methanol is eliminated from **2** to give the corresponding indolohydroquinone **4**.[6] This same type intermediate can be generated from aziridinomitosenes such as **3**, and this kind of mitomycin analogue also has good antitumor activity.[7] (7-Methoxymitosenes have about the same quinone reduction potential as mitomycin C.)

In principle, 1-substituted mitosenes (e.g., **6**) should undergo bioreduction to indolohydroquinones that are able to give bifunctional alkylation and cross-linking of DNA, provided that the 1-substituent is a suitably good leaving group. This idea had been proposed by Allen et al. in 1967 as a means of improving the antibacterial activity of 2-substituted indoloquinones related to the mitomycins.[8] However, it was not valid in that respect. Nevertheless, our studies on the synthesis and biological activities of mitosenes indicated that the idea might be valid for antitumor activity. Thus, of four 1-substituted 7-methoxymitosenes tested in the P388 mouse leukemia assay, the 1-acetoxy derivative **6** was active, whereas the 1-hydroxy and 1-(*N*-pyrrolidino) derivatives **7** and **8** were not.[9] The quaternary ammonium compound **9** was inactive despite having a good leaving group at position 1; however, it was likely that this compound had poor cell penetration.[9] These results suggested that additional 1-substituted mitosenes with good, nonionic leaving groups should be prepared and tested to substantiate the idea of bifunctional alkylation. Consequently, a series of esters and carbamates based on the 1-acetoxymitosene **6** was undertaken. Other leaving groups of interest included halides and sulfonates. We also planned to covert a few of the more active analogues into their 7-(1-aziridinyl) derivatives because this type of replacement enhanced the antitumor activity of compounds in both the mitosane (mitomycin C related)[10] and aziridinomitosene groups.[7]

Chemistry. 1-Hydroxy-7-methoxymitosene (**7**) was the key intermediate for the preparation of the kinds of analogues proposed above. This compound had been synthesized by us previously,[11] but the route was difficult and in need of improvement if useful amounts of the analogues were to be obtained. The basic scheme for the synthesis was retained. However, many individual steps were changed, with resulting simplification in operation and improvement in yield. 2,5-Dimethyl-4-nitroanisole (**10**) was prepared as described previously and converted into the phenylpyruvic acid derivative **12** by way of the methyl ester **11** (Scheme II).[12] Conversion of **12** into the indole-2-carboxylic acid ester **14** was accomplished by a variation of the known method involving a reductive cyclization with ferrous ammonium sulfate followed by Fischer esterification.[12] The workup of this reduction mixture was greatly simplified by centrifuging to separate the ferric oxide sludge that previously required washing with very large quantities of ammonium hydroxide (see Experimental Section). A new method based on direct reductive cyclization of **11** to **14** in the presence of tin and ethanolic hydrogen chloride was established on a small scale. It gave a 50% yield, which is competitive with the more difficult alternative route (42–50%). However, the new route has not yet been proven on a large scale.

The conversion of **14** into 1-acetoxypyrrolo[1,2-*a*]indole **16** was completed by the previously described method,[12] except that the purification of the 1-hydroxy intermediate **15** and its acetylation were improved (Experimental Section). Vilsmeier–Haack formylation of **16** to give **17**[11] was modified by using methylene chloride and pyridine as cosolvents. This change resulted in reproducible yields of about 95%. The 8-nitro derivative **18**, whose purification was improved by chromatography instead of recrystallization, was reduced to the corresponding amine **19** by a new procedure involving tin in ethanolic hydrogen chloride.

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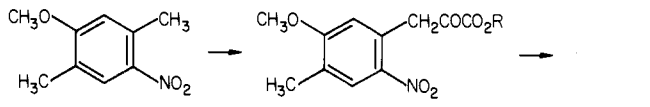
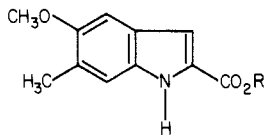
Scheme II

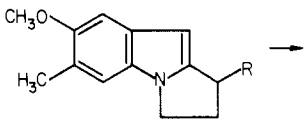


10

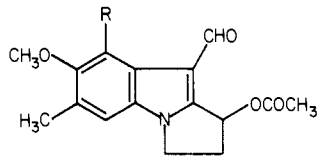
11, R = CH_3
12, R = H



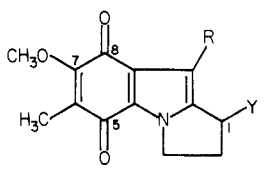
13, R = H
14, R = CH_3



15, R = OH
16, R = $OCOCH_3$



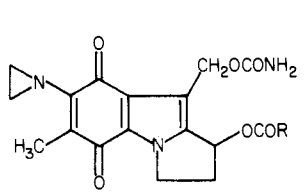
17, R = H
18, R = NO_2
19, R = NH_2



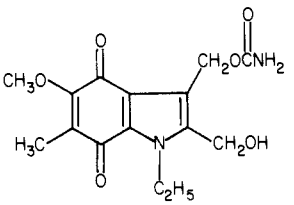
20, R = CHO; Y = $OCOCH_3$
21, R = CH_2OH; Y = $OCOCH_3$
22, R = CH_2OH; Y = H
23, R = CH_2OCONH_2; Y = H
24, R = CH_2OCONH_2; Y = $OCONH_2$
25, R = CH_2OCONH_2; Y = $OCONHCH_3$
26, R = CH_2OCONH_2; Y = OCO_2CH_3
27, R = CH_2OCONH_2; Y = $OCOC(CH_3)_3$
28, R = CH_2OCONH_2; Y = $OCOCH_2Cl$

29, R = CH_2OCONH_2; Y = 

30, R = CH_2OCONH_2; Y = Cl
31, R = CH_2Cl; Y = Cl
32, R = CH_2Cl; Y = OH
33, R = CH_2OH; Y = OH
34, R = CH_2Cl; Y = $OCONHCH_3$
35, R = CH_2OCONH_2; Y = =NOH



36, R = CH_3
37, R = NH_2



38

This amine could be isolated, but it usually was converted directly into the quinone **20** by potassium nitrosodisulfonate (Fremy's salt) in acetone–phosphate buffer. Yields up to 82% for the preparation of **20** from **18** were obtained on a gram scale and they represent a substantial improvement over the previous method (39%).[11]

The next step in the synthesis, reduction of the 9-formyl group to the corresponding alcohol, was initially the most difficult and nonreproducible one. The previously reported method,[11,12] involving sodium borohydride in ethanol, results in reduction of the quinone to an unstable hydroquinone that must be reoxidized (with $FeCl_3$) before workup. In our hands, yields of the product **21** ranged from 35 to 65%, and a second product (**22**), resulting from 1-deacetoxylation, was present in small amounts. The formation of **22** from the hydroquinone of **20** can be visualized as a process involving simultaneous loss of the 1-acetoxyl and a proton from the 8-hydroxyl group, followed by addition of a proton at C-1 and loss of a proton from the 5-hydroxyl group. In order to overcome these difficulties of low yield and byproduct, we decided to seek a reagent that would selectively reduce the aldehyde group of **20** over its quinone ring. 9-Borabicyclo[3.3.1]nonane (9BBN) was an attractive possibility because its large size suggested selective approach to the aldehyde group. In fact, it showed a high degree of chemoselectivity when limited quantities were used. Thus, reduction of **20** in tetrahydrofuran with 2 equiv of 9BBN gave reproducible yields of about 70% for **21**, with no **22** detectable.

Samples of **21** prepared by 9BBN method could be converted directly into the corresponding carbamate **6** by the usual method of phenyl chloroformate, followed by liquid ammonia.[12] Hydrolysis of the 1-acetoxy group to the 1-ol **7** was effected by ammonium hydroxide in methanol[11] or, better, by methanolic potassium hydroxide. For samples of **21** contamined with the 1-deacetoxylated product **2**, it was more convenient to carry the mixture through to the 1-hydroxy-7-methoxymitosene (**7**) stage and then separate this product from the 7-methoxymitosene (**23**).

The preparation of 7-methoxymitosenes **24–29** with new esters at position 1 was accomplished by treating **7** with appropriate acid chlorides, anhydrides, and isocyanates. We were not able to prepare the corresponding trifluoroacetate, methanesulfonate, or toluenesulfonate derivatives because of their instability. In the case of methanesulfonyl chloride in pyridine, a water-soluble product was formed. This product was not isolated and characterized, but it might have been the 1-pyridinium derivative by analogy to the product formed when a related 2-(hydroxymethyl)indoloquinone (**38**) was treated under the same conditions.[8]

Attempts to convert 1-hydroxymitosene **7** into the corresponding 1-chloro derivative **30** gave some unexpected results. Treatment with thionyl chloride or phosphorus oxychloride in the presence of a variety of bases resulted only in decomposition. In contrast, this type of conversion went readily with indoloquinone **38**.[8] When we tried an alternative approach involving treatment of **6** with anhydrous hydrogen chloride in methylene chloride and acetic anhydride, followed by chromatography on silica gel with methylene chloride–acetone (9:1) as solvent, the product was identified as the 10-(chloromethyl)-1-hydroxy derivative **32**. It seems likely that the 1-hydroxyl group had been formed from the 1-chloro substituent on chromatography, particularly since chromatography of a similar reaction product on a column of freshly dried silica gel gave a main spot with a much higher R_f value. Unfortunately, the component of this spot (presumably **31**) was too unstable for isolation and characterization. If chromatography of the reaction product was done on a moistened silica gel column, the 10-(hydroxymethyl)-1-hydroxy derivative **33** was obtained. These experiments give a rough indication of the relative reactivity of substituents at the 1 and 10 positions toward nucleophiles. It was possible to prepare the methyl carbamate **34** by treating **32** with methyl isocyanate.

Some miscellaneous structures also were prepared. The 1-oxime **35** was obtained by manganese dioxide oxidation of **7**, followed by treatment with hydroxylamine. Our interest in **35** was based on good antibacterial activity of

Table I. Activity of Mitosenes against P388 Murine Leukemia[a]

compd	max effect: % T/C, compd (mito C)	opt dose mg/kg	MED, mg/kg	TR, opt dose/ MED
6	165 (229)	6.4	0.8	8
7	113 (181)	6.4		
23	128 (183)	51.2	51.2	1
24	163 (238)	6.4	0.4	16
25	167 (228)	6.4	1.6	4
26	150 (244)	3.2	1.6	2
27	117 (183)	51.2		
28	111 (224)	6.4		
29	128 (211)	6.4	6.4	1
34	139 (222)	12.8	12.8	1
35	150 (181)	6.4	1.6	4
36	122 (224)	6.4		
37	144 (183)	51.2	0.8	64
3	213 (181)	12.8	0.8	16

[a] Determined at Bristol Laboratories, Syracuse, NY. A tumor inoculum of 10^6 ascites cells was implanted in CDF_1 female mice. Six mice were used at each dose of the compound, given on day 1 only, and 10 control mice were injected with saline. A control group of six mice at each dose in each experiment received mitomycin C. The optimal dose of mitomycin C usually is 3.2 mg/kg and its minimum effective dose is 0.2. MST = median survival time; max effect (96 T/C) = MST treated/MST control × 100 at the optimal dose; MED = minimum effective dose (% T/C = 125); TR = therapeutic ratio (opt dose/MED). There were no 30-day survivors among mice treated with the 1-substituted mitosenes. However, mitomycin C showed one or more survivors in certain experiments. The complete testing data, including for each dose used the therapeutic effect, number of 5-day survivors, and average weight difference between the test and control group, are available as supplementary material (see paragraph at the end of paper).

Table II. Activity of Mitosenes against L1210 Murine Leukemia[a]

compd	dose, mg/kg	effect MST: % T/C	av wt change, g
24	24	toxic	−5.1
	12	122	−3.4
NSC 338266	6	116	−1.7
	3	118	−3.2
	1.5	114	−1.4
37	24	138	−3.2
	12	134	−2.4
NSC 338267	6	114	−1.6
	3	112	−1.1
	1.5	108	−0.6
1	12	110	−5.5
mito C	6	124	−3.6
NSC 026980	3	122	−2.4
	1.5	108	−1.2

[a] Determined at Arthur D. Little, Cambridge, MA. A tumor inoculum of 10^6 ascites cells was implanted into BDF_1 female mice. Six mice were used at each dose of the mitosene, which was given on day 1 as a single injection in distilled water containing Tween 80. All groups had six survivors on day 5, except at the highest doses of 24 and 1, where there were three and five survivors, respectively. There were no 30-day survivors. MST = median survival time in days; % T/C = MST treated/MST control × 100; significant effect = 125. Average weight change is the average weight difference between the treated and control groups. The control group contained 36 mice.

a related compound in the indoloquinone series.[8] 7-(1-Aziridinyl) analogues **36** and **37** were prepared from **6** and **24**, respectively, by treatment with ethylenimine in methanol.

Biological Activity. The activities of the 1-substituted mitosenes against P388 leukemia in mice are given in Table I. Although the assays were not all performed concurrently, each analogue was standardized against mitomycin C in the same experiment. The maximum effect on life span (% T/C) for mitomycin C at its optimal dose is given in parentheses beside the maximum value for each test compound. Because of the usual variations among experiments, compounds should not be compared directly with each other but according to their effectiveness relative to mitomycin C. Only the optimal dose, minimum effective dose (% T/C ≥125), and therapeutic ratios are given in Table I. The complete series of doses with their effects on life spans and the weight differences between test and control animals are given as supplementary material (see the paragraph at the end of this article).

The most important result in Table I is that a leaving group at position 1 appears to be necessary for significant antitumor activity. Compounds such as **6**, **24**, and **25** with acetoxy, carbamoyloxy, and methylcarbamoyloxy groups, respectively, have moderate activity and therapeutic ratios. In contrast, compound **7** with a hydroxyl group is inactive, and the 7-unsubstituted analogue **23** is barely active at a relatively high dose. As previously mentioned, the 1-(*N*-pyrrolidinyl) analogue also was inactive.[9] The methyl carbonate **26** and nicotinate **29** were active, but the pivalate **27** and chloroacetate **28** were not. It is difficult to give a definitive interpretation of these observations because a number of factors are involved in activity. Thus, the inactivity of **27** might be due to steric factors and/or excess lipophilicity. The chloroacetoxy group of **28** certainly is a stronger leaving group than most of the others, but it fails to confer activity. It might be that this group is too reactive and is lost to hydrolysis or nonspecific alkylation before it can reach the DNA of the leukemia cells. The 1-oximino analogue **35** showed moderate activity against P388 leukemia. This result appears to contradict the requirement for a good leaving group at the 1 position. It is possible that the oxime undergoes metabolism to a leaving group, but there is no evidence on this point.

The effects produced by substituting a 7-(1-aziridinyl) group for the 7-methoxy group are difficult to explain. Activity against P388 leukemia is not enhanced in the 1-acetoxy compound **36** or the 1-(carbamoyloxy) compound **37**, although the latter compound did have a good therapeutic ratio. However, compound **37** showed activity against L1210 leukemia at two different dose levels, whereas the corresponding 7-methoxy analogue **24** was inactive in this assay (Table II). The mitomycin C standard also was not active in this particular assay, although it generally shows T/C of 125–161 at one or two dose levels. Compound **37** is the only mitomycin analogue without a 1,2-aziridino group to ever show activity against L1210 leukemia.[10]

Comparison of the 1-substituted mitosenes described above with the related aziridinomitosene **3**[7] reveals the latter to be much better in prolonging life in the P388 assay, although it is not more potent. This observation suggests an additional selectivity factor for cancer cells over normal cells that is inherent in the structure and properties of **3**. On the reasonable assumption that this factor is the activation of the aziridine ring for alkylation by protonation[13] and knowing that some cancer cells have lower pH than normal cells,[14] we decided to prepare a 1-substituted

(13) Lown, J. W.; Begleiter, A.; Johnson, D.; Morgan, A. R. *Can. J. Biochem.* **1976**, *54*, 110.

mitosene in which the substituent would be activated as a leaving group by protonation. The nicotinyl ester **29** was chosen for this purpose because its anticipated pK_a ($\sim$3.1)[15] is near to that of the aziridine ring in mitomycin C.[16] Unfortunately, **29** does not show as much activity as simple esters such as **6** (Table I). This result does not necessarily disprove the concept of activation by protonation, because the nicotinyl group might provide unfavorable size or lipophilicity. However, it has limited our enthusiasm to prepare other analogues of this type.

Experimental Section

Melting points were determined on a Laboratory Instruments Mel-Temp apparatus and are uncorrected. IR spectra were taken on a Beckman IR-33 spectrometer with samples prepared as potassium bromide pellets. Absorptions are reported in reciprocal centimeters. NMR spectra were taken on a Varian EM-360L 60 MHz spectrometer and absorptions are reported as parts per million downfield from Me_4Si. Mass spectral data were obtained on a Varian 311A instrument with an ionizing voltage of 70 eV and a source temperature of 250 °C. Samples were introduced by direct probe inlet. Reported are the molecular ion followed by the most intense ion unless otherwise indicated. Elemental analyses were performed either by the University of Arizona Analytical Center or Galbraith Laboratories, Inc. Thin-layer chromatography (TLC) was carried out using a commercially prepared 2-mm layer of silica gel 60 on plastic sheets. The phrase "chromatography on silica gel" denotes the use of silica gel 60 (70–270 mesh) with gravity elution. The acronym "LPC" (low-pressure chromatography) indicates the use of MN-Silica Gel P, intended for preparation of TLC plates, packed in a column, and elution was accomplished at 2–3 psi (pressure generated by an argon cylinder). Adsorbents for these three forms of chromatography were purchased from Brinkmann Instruments, Inc. Preparative scale high-performance liquid chromatography (HPLC) was carried out on a Waters Associates Preparative 500 instrument using a single Waters PrepPAK-500 silica column. Semipreparative scale HPLC was performed using a Spectra-Physics Model 3500 liquid chromatograph and a Whatman Partisil M9 10/50 column.

5-Methoxy-6-methyl-2-indolecarboxylic Acid (13). A mechanically stirred solution of ferrous sulfate heptahydrate (483 g, 1.74 mol) and water (550 mL) was heated to 85 °C and treated slowly with a solution of the crude pyruvic acid derivative, **12** (60.3 g, 0.24 mol), in 11.5% ammonium hydroxide (500 mL). After the addition was complete, the mixture was stirred at 85–100 °C for 1 h and cooled to room temperature. Centrifugation separated a black solid from a brown solution. The solution was decanted, filtered, and acidified with 37% hydrochloric acid to precipitate **13**. The black solid was then successively suspended in 11.5% ammonium hydroxide and recentrifuged until the filtered supernatant yielded no product upon acidification. The combined indolecarboxylic acid, **13**, was collected by filtration, washed well with water, and dried at reduced pressure to give 32.3 g (66%) as a tan solid: mp 238–241 °C (decomposes); IR 3390, 3100–2900, 1665, 1520 cm^{-1}; TLC (chloroform–methanol, 4:1) of this material was identical with an authentic sample.[11]

Methyl 5-Methoxy-6-methyl-2-indolecarboxylate (14). Method 1. A mixture of **13** (33 g, 0.16 mol), dry methanol (900 mL), and concentrated sulfuric acid (14 mL) was stirred first at reflux for 4 h and then at room temperature overnight. The excess methanol was evaporated at reduced pressure and the resulting solid was dissolved in chloroform (800 mL). Extraction with 5% aqueous sodium bicarbonate (4 × 250 mL) removed some starting material, which was recovered by acidification of the aqueous fractions and filtration: 4.6 g (14%) of **13** was recovered. The organic phase was dried over magnesium sulfate and evaporated to a concentrated solution, which was chromatographed on silica gel with chloroform. Collection of the major, pale-colored band gave **14** (22.5 g, 64%) as a white, crystalline solid: mp 144–146 °C; IR 3340, 1687 cm^{-1} (lacks COOH absorption of **13**); NMR ($CDCl_3$) δ 7.15 (s, 2 H), 6.90 (s, 1 H), 3.92 (s, 3 H), 3.85 (s, 3 H), 2.30 (s, 3 H); TLC (methylene chloride–hexane, 3:1) of this material was identical with an authentic sample.[11]

Method 2. A solution of **13** (65 g, 0.32 mol) in dry methanol (1.8 L) was bubbled with a slow stream of anhydrous hydrogen chloride at reflux for 24 h. It was then flushed with nitrogen and cooled to room temperature. Dilution with 5% sodium bicarbonate saturated with sodium chloride (2.5 L) precipitated the product, **14**, which was collected by filtration, washed well with water, and dried at reduced pressure to give 53 g (76%) as a tan solid. Recrystallization of several combined batches from methanol gave material of identical purity and spectral properties to that produced by method 1.

Method 3. A solution of crude **12**[12] (1.38 g, 5.4 mmol), methanol (60 mL), and toluene (60 mL) was treated with concentrated sulfuric acid (0.6 mL) and stirred at reflux temperature for 12 h using a Sohxlet extractor filled with dry 3Å molecular sieves to trap water produced by the reaction. After cooling in an ice bath, the reaction mixture was diluted with chloroform (150 mL) and repeatedly washed with water until a test sample of the aqueous layer was neutral. The organic fraction was treated with magnesium sulfate and activated charcoal (ca. 2 g each), filtered, and evaporated at reduced pressure. The residual solid was chromatographed on silica gel with methylene chloride–hexane (5:1) to give **11** (970 mg, 70%) as a nearly white solid: mp 111–113 °C; IR 1700, 1730 cm^{-1}; NMR ($CDCl_3$) δ 8.02 (s, 1 H), 6.63 (s, 1 H), 4.46 (s, 2 H), 3.97 (s, 6 H), 2.27 (s, 3 H).

A suspension of **11** (200 mg, 0.75 mmol), ethanol (70 mL), and tin metal (800 mg, 30-mesh granules) was treated with 3 N hydrochloric acid (16 mL) and stirred for 1 h at room temperature. The resulting solution was decanted from excess tin, neutralized with saturated aqueous sodium bicarbonate, and extracted with chloroform (2 × 100 mL). The combined organic fractions were dried over magnesium sulfate, concentrated at reduced pressure, and chromatographed on silica gel with methylene chloride–hexane (3:1) to give **14** (88 mg, 50.8%) as a white solid: mp 145–147 °C; IR and NMR were identical with the product of method 1.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole (16). The tricyclic alcohol **15**[11] (2.7 g, 12.4 mmol) was dissolved in pyridine (60 mL) and treated with acetic anhydride (40 mL). The mixture was stirred for 16 h at room temperature, poured into ice–water (500 mL), and stirred for 15 min before collecting the resulting precipitate by filtration. The solid was dissolved in methylene chloride, washed well with water, dried over magnesium sulfate, and evaporated at reduced pressure to give **16** (2.9 g, 90%) as a white solid: mp 131–132 °C (lit.[11] 134–135 °C); IR 1727 (lacks 3500–3200 OH absorption of **15**); NMR ($CDCl_3$) δ 7.01 (s, 1 H), 6.97 (s, 1 H), 6.30 (s, 1 H), 6.10 (d of d, 1 H), 4.12 (m, 2 H), 3.85 (s, 3 H), 2.81 (m, 2 H), 2.35 (s, 3 H), 2.05 (s, 3 H).

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (17). DMF was dried over potassium hydroxide and distilled immediately prior to use in both procedures.

Method A. Prepared According to the Literature Method.[11] From 1.7 g of **16** was obtained 1.35 g (72%) of **17** as nearly white solid, mp 166–168 °C.

Method B. A mixture of methylene chloride (5 mL) and DMF (2 mL, 25.8 mmol) was treated at 5 °C with phosphorous oxychloride (1.2 mL, 13.1 mmol, freshly distilled) and stirred for 15 min under argon. This mixture was then treated with a solution composed of **16** (3.0 g, 11.6 mmol), dry pyridine (5 mL, 62.1 mmol), and methylene chloride (55 mL). The reaction was stirred at 5 °C for 50 min, treated with cold saturated sodium acetate (225 mL), and stirred vigorously for 4 h. The orange organic phase was separated from the red aqueous phase and washed with 1.5 N hydrochloric acid (3 × 200 mL), followed by water (1 × 200 mL). After drying over magnesium sulfate, the solvent was evaporated to give **17** (3.24 g, 97.5%) as an off-white solid, which was identical in IR and NMR spectra with the sample produced by method A.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (18). A mixture of **17** (3.0 g, 11.6 mmol) and glacial acetic acid (50 mL) was treated with

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(15) *Beilstein's Handb. Org. Chem.* 1979, *22*(1), 357.
(16) Tomasz, M.; Lipman, R. *J. Am. Chem. Soc.* 1979, *101*, 7121.

90% nitric acid (3.0 mL) and stirred for 30 min at room temperature. It was then poured into ice–water (600 mL) and stirred for a few minutes before the precipitate was collected by filtration, washed well with water, dissolved in chloroform, extracted with water, dried over magnesium sulfate, and evaporated at reduced pressure. The crude nitro compound **18** was purified by chromatography on silica gel with chloroform to give 3.3 g (85%) as a pale yellow solid: mp 176–178 °C (lit.[11] 175–176 °C); IR 1750, 1665, 1535, 1380 cm^{-1}; NMR ($CDCl_3$) δ 9.73 (s, 1 H), 7.20 (s, 1 H), 6.33 (d of d, 1 H), 4.20 (t, 2 H), 3.77 (s, 3 H), 3.00 (m, 2 H), 2.34 (s, 3 H), 200 (s, 3 H); TLC (chloroform) was identical with an authentic sample.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-5,8-dioxo-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (20). Finely ground **18** (1.0 g, 3.0 mmol) was suspended in ethanol (250 mL) and treated with tin metal (3.2 g, 30-mesh granules) and 3 N hydrochloric acid (68 mL). The mixture was stirred vigorously for 70 min, and the resulting clear solution was decanted from excess tin. Saturated aqeuous sodium bicarbonate was added dropwise until a white precipitate formed, and the mixture was then extracted with chloroform to remove a yellow organic fraction, which was washed with water, dried over magnesium sulfate, and evaporated to give the amine **19** as a thick yellow oil, which was used without purification in the next step: IR 3440, 3320, 2940 1745, 1645 cm^{-1}; NMR ($CDCl_3$) δ 9.60 (s, 1 H), 6.25 (m, 2 H), 5.67 (broad, 2 H), 4.00 (t, 2 H), 3.73 (s, 3 H), 2.80 (m, 2 H), 2.33 (s, 3 H), 2.05 (s, 3 H).

The amine from above was dissolved in acetone (180 mL) and treated with a solution of potassium nitrosodisulfonate (4.5 g) in 0.3 M, pH 6.0 phosphate buffer (180 mL). The reaction mixture was stirred for 2 h, diluted with water, and extracted with methylene chloride to give an orange organic fraction and a purple aqueous fraction. The organic fraction was washed with 5% sodium carbonate (2 × 200 mL) and water (1 × 200 mL), then dried over magnesium sulfate, and concentrated. The residue was immediately purified on silica gel by eluting with chloroform to give an orange oil that precipitated upon evaporation from acetone–hexane at reduced pressure to give **20** (777 mg, 81.7%) as a bright orange solid: mp 139–140 °C; IR 3020–2860, 1740, 1680, 1660, 1640 cm^{-1}; NMR ($CDCl_3$) δ 10.33 (s, 1 H), 6.32 (d of d, 1 H), 4.29 (m, 2 H),4.06 (s, 3 H), 2.90 (m, 2 H), 2.10 (s, 3 H), 1.99 (s 3 H). This material was identical in spectral and TLC (chloroform) properties with that produced by established procedures, but its melting point is higher (lit.[11] 126–127 °C). Occasionally the product required additional purification in order to obtain a solid. This could be accomplished by preparative HPLC, eluting with methylene chloride–acetone (95:5) at 250 mL/min. The impurity was a small quantity of an unidentified yellow oil with a slightly longer retention time.

1-Acetoxy-2,3-dihydro-9-(hydroxymethyl)-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (21). Method 1. A solution of ethanol (40 mL, dried at reflux over magnesium and distilled), tetrahydrofuran (20 mL, dried over lithium aluminum hydride and distilled), and glacial acetic acid (0.05 mL) was repeatedly evacuated and repressurized with argon to remove all oxygen. It was then treated with sodium borohydride (350 mg, 9.2 mmol), and after bubbling stopped, the system was again flushed with argon. Finally, solid **20** (350 mg, 1.1 mmol) was added, and the system was flushed and stirred under a stream of argon until the orange color faded to a nearly colorless solution (ca. 10 min.). Acetone (10 mL), which had been bubbled with argon to remove air, was added and the reaction mixture was stirred for 2 min before adding dropwise 1 N ferric chloride in 0.1 N aqueous hydrochloric acid (10 mL). The mixture was diluted with water and methylene chloride, the organic phase was separated, and the aqueous layer was washed with methylene chloride until no orange color was extracted. The combined organic fractions were washed with water, dried over magnesium sulfate, concentrated at reduced pressure, and chromatographed on silica gel with chloroform. Collection of the major orange band and evaporation of solvent gave impure **21** (225 mg) as an orange solid: mp 112–113 °C (lit.[11] 123–124 °C); IR 3420, 1730, 1640 cm^{-1}; NMR ($CDCl_3$) δ 6.01 (d or d, 1 H), 4.67 (s, 2 H), 4.30 (t, 2 H), 4.00 (s, 3 H), 2.75 (m, 2 H), 2.09 (s, 3 H), 1.97 (s, 3 H). TLC (methylene chloride–acetone, 95:5) showed a minor red-orange spot just above the major orange spot. Purification of two or three combined batches by preparative HPLC, eluting with methylene chloride–acetone (95:5) at 250 mL/min, easily separated this minor component which has the spectral characteristics of **22**: NMR ($CDCl_3$) δ 4.58 (s, 2 H), 4.20 (m, 2 H), 4.00 (s, 3 H), 2.65 (m, 4 H), 2.00 (s, 3 H); MS, *m/e* 261, 246. This separation was not routinely carried out, since after further steps in the synthesis the mixture of **6** and **23** was easily separated by gravity chromatography.

Method 2. A solution of **20** (200 mg, 0.63 mmol) in tetrahydrofuran (7.5 mL, freshly dried over lithium aluminum hydride and distilled) was chilled under nitrogen to −10 °C and treated with 0.5 M 9-borabicyclo[3.3.1]nonane in tetrahydrofuran (2.6 mL, 1.3 mmol). The reaction was stirred under nitrogen as it slowly warmed to room temperature over 20 min. It was then returned to the ice bath and treated with 30% hydrogen peroxide (10 mL, 1 mL at a time). After stirring for several minutes, the mixture was diluted with water and extracted with methylene chloride. The organic fraction was washed with water, dried over magnesium sulfate, and concentrated at reduced pressure to a dirty orange residue. It was then immediately purified by chromatography on silica gel after first adsorbing on a small quantity of silica gel from acetone solution. The elution solvent used for chromatography was methylene chloride–acetone (95:5). The major orange band was collected, and the solvent was removed at reduced pressure to give **21** (144 mg, 71.5%) as an orange solid. This material was identical in spectral and TLC (methylene chloride–acetone 9:1) properties with that prepared by method A, except that no **22** was visible on the TLC chromatogram. It was identical in IR and NMR spectra with an authentic sample of pure **21**.[11]

1-Hydroxy-7-methoxymitosene (7) and 7-Methoxymitosene (23). Method 1. A 150-mg sample of **6**, containing a small amount **23**, was prepared from the mixture of **21** and **22** according to the literature method.[11] It was dissolved in methanol (175 mL), treated with 30% ammonium hydroxide (5 mL), and stirred for 10 h at room temperature. The methanol was removed at reduced pressure and the residue was dissolved in methylene chloride for purification on silica gel with methylene chloride–acetone (4:1) as eluent. A small orange band eluted ahead of the major orange band (not seen when the 9BBN method was used earlier in the synthesis to prepare **21**). Collection of the former and evaporation of solvent gave **23** (5 mg, 6% of the product) as an orange-red solid: IR 3410, 3300, 1685, 1640 cm^{-1}; NMR ($CDCl_3$) δ 5.22 (s, 2 H), 4.65 (broad, 2 H), 4.18 (t, 2 H), 4.00 (s, 3 H), 2.70 (m, 4 H), 1.95 (s, 3 H); TLC (methylene chloride–acetone, 6:1) was identical with an authentic sample.[12] Collection of several batches and recrystallization from ethanol gave orange needles: mp 205–206 °C (lit.[12] 206–207 °C). The major orange band gave **7** (103 mg, 77.7%) as an orange solid upon evaporation of solvent: mp 189–190 °C (lit.[11] 195–196 °C); IR 3400, 1685, 1640 cm^{-1}; NMR (Me_2SO-d_6) δ 6.10 (broad, 2 H), 5.12 (s, 3 H), 4.19 (t, 2 H), 3.93 (s, 3 H), 3.20 (s, H_2O), 2.50 (m, 2 H), 1.90 (s, 3 H); TLC (methylene chloride–acetone, 4:1) was identical with an authentic sample.

Method 2. A solution of **6** (200 mg, 0.55 mmol) in methanol (250 mL) was treated with 1.0 N potassium hydroxide (1.6 mL) and stirred for 3 h at room temperature. The solution was then acidified with 1.0 N hydrochloric acid (1.5 mL), and the methanol was evaporated at reduced pressure. The residue was adsorbed on silica gel by evaporation from methylene chloride and then it was purified by chromatography on silica gel, eluting with methylene chloride–acetone (4:1). Collection of the major orange band and evaporation of solvent gave **7** (159 mg, 89.9%) as an orange solid that was identical with that produced by method 1.

1-Hydroxy-7-methoxymitosene Carbamate (24). A solution of **7** (110 mg, 0.36 mmol) in pyridine (25 mL) and methylene chloride (50 mL) was chilled to 0 °C, treated with phenyl chloroformate (2.5 mL, 10.5 mmol), and stirred overnight at room temperature. The mixture was chilled in an ice bath and diluted with water. It was then extracted with methylene chloride, and the organic fraction was washed with 3 N hydrochloric acid (4 × 50 mL) and water (1 × 100 mL), dried over magnesium sulfate, and evaporated at reduced pressure. Chromatography of the resulting oil on silica gel with toluene removed some diphenyl carbonate. Elution with chloroform then gave one major orange band and several lesser orange bands. Collection of the major band and evaporation of solvent gave the phenyl carbonate derivative as an orange oil, which was difficult to dry as a solid:

NMR ($CDCl_3$) δ 7.25 (m, 5 H), 6.3 (d of d, 1 H), 5.33 (s, 2 H), 4.89 (broad, 2 H), 4.39 (t, 2 H), 4.03 (s, 3 H), 2.85 (m, 2 H), 1.97 (s, 3 H).

The oil from above was dissolved in 50 mL of methylene chloride and chilled on a dry ice–acetone bath while anhydrous ammonia was bubbled into it. When the volume reached about 65 mL the bubbling was stopped, the flask was removed from the bath, and the contents were slowly warmed to room temperature over 3 h while stirring. After the ammonia had boiled away, the resulting mixture was evaported to a sticky solid, which was adsorbed on a small quantity of silica gel by stirring in methylene chloride and evaporating to dryness at reduced pressure. The adsorbed product was purified by chromatography on silica gel, eluting with methylene chloride–methanol (95:5) to obtain **24** (60 mg, 48.1%) as a yellow solid upon evaporation of solvent from the major yellow band: mp 200 °C dec; IR 3460–3300, 1680, 1640 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) 6.30 (broad, 2.5 H), 6.07 (broad, 2.5 H), 5.13 (s, 2 H), 4.20 (m, 2 H), 3.97 (s, 3 H), 3.30 (s, H_2O), 2.55 (m, Me_2SO), 1.90 (s, 3 H); MS, *m/e* 363, 259.

Repeated attempts to obtain an elemental analysis gave results inconsistent with the molecular formula despite the overwhelming spectral evidence supporting the designated structure. Hence, **24** was purified by semipreparative scale HPLC as follows: A solution of **24** (7 mg) in DMF–methylene chloride (1:1, 3.5 mL) was chromatographed (ca. 0.4 mL per run) by eluting first with methylene chloride for 3 min and then introducing a 0–5% linear gradient of methanol over 20 min. Elution rate was 5 mL/min. Some nonpolar material eluted first, followed by DMF. Next to elute were a major yellow fraction and four minor components. Center cuts of the major component were collected for each run and combined. A portion (0.5 mL) was then injected and eluted as above to give a single sharp peak on the recorder chart, indicating pure **24**. Evaporation of solvent gave **24** as a slightly sticky yellow solid, which was triturated with acetone and dried at room temperature under a vacuum for 48 h. The microanalysis for C, H, and N was consistent with $C_{16}H_{17}N_3O_7 \cdot (CH_3)_2CO$. A high-resolution mass spectrum gave a molecular ion at 363.1067. $C_{16}H_{17}N_3O_7$ requires 363.1068.

1-Hydroxy-7-methoxymitosene Methylcarbamate (25). A suspension of potassium carbonate (2 g) in methylene chloride was treated with methyl isocyanate (1 mL, 16.2 mmol) and stirred for 5 min before adding a solution of **7** (21 mg, 0.06 mmol) in methylene chloride (10 mL). The reaction was stoppered and stirred at room temperature for 15 h. It was then filtered and the potassium carbonate was rinsed with chloroform and ethyl acetate until colorless. The combined organic fraction was evaporated at reduced pressure and chromatographed on silica gel after first adsorbing on about 1 g of the gel. Elution with chloroform–ethanol (98:2) gave two yellow bands. The smaller band eluted first. It was evaporated to a small quantity of an unidentified oil. The second, larger band gave **25** (12.8 mg, 51.7%) as a bright yellow solid: mp 225 °C (decomposes); IR 3460, 3300, 1680, 1640 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 6.20 (m, 1 H), 5.15 (s, 2 H), 4.92 (broad, 2 H), 4.73 (broad, 1 H), 4.20 (t, 2 H), 3.91 (s, 3 H), 2.85–2.50 (m, 5 H), 1.75 (s, 3 H); MS, *m/e* 377, 259. Anal. ($C_{17}H_{19}N_3O_7$) C, H, N.

1-Hydroxy-7-methoxymitosene Methyl Carbonate (26). A solution of **7** (50 mg, 0.16 mmol), methylene chloride (20 mL), and pyridine (5 mL) was treated at 0 °C with methyl chloroformate (1 mL, 12.9 mml). The reaction was stirred at room temperature for 24 h and then diluted with water. Extraction with methylene chloride removed an orange organic fraction, which was washed with 3 N hydrochloric acid (4 × 50 mL) and water (1 × 100 mL), dried over magnesium sulfate, and evaporated. The concentrated residue was purified by LPC, eluting with chloroform–acetone (95:5) to give an orange solid that was difficult to dry. Recrystallization from ethanol afforded **26** (27 mg, 45.7%) as a crystalline orange solid: mp 187–190 °C; IR 3440, 3330, 3260, 1730, 1640 cm^{-1}; NMR ($CDCl_3$) δ 6.16 (d of d, 1 H), 5.25 (s, 2 H), 4.80 (broad, 2 H), 4.27 (t, 2 H), 3.93 (s, 3 H), 3.75 (s, 3 H), 2.71 (m, 2 H), 1.85 (s, 3 H); MS, *m/e* 378, 259. Anal. ($C_{17}H_{18}N_2O_8$) C, H, N.

1-Hydroxy-7-methoxymitosene Trimethylacetate (27). A suspension of potassium carbonate (3 g) in ethyl acetate (2 mL) was treated with trimethylacetyl chloride (1.7 mL, 13.8 mmol) and stirred for 5 min before adding a solution of **7** (24.5 mg, 0.08 mmol) in ethyl acetate (10 mL). The reaction was stirred under argon for 4 days and then diluted with water and ethyl acetate. The organic fraction was washed with water, dried over magnesium sulfate, and evaporated at reduced pressure to give an orange solid suspended in a high-boiling liquid (excess acid chloride). This mixture was dissolved in chloroform and purified by LPC, using chloroform as the eluent. Evaporation of solvent from the major band gave **27** (13.3 mg, 43.0%) as an orange solid: mp 238–239 °C dec; IR 3440, 3320, 3270, 1720, 1640, 1630 cm^{-1}; NMR ($CDCl_3$) δ 6.33 (m, 1 H), 5.27 (s, 2 H), 4.78 (broad, 2 H), 4.30 (t, 2 H), 4.00 (s, 3, H), 2.75 (m, 2 H), 1.97 (s, 3 H), 1.20 (s, 9 H); MS, *m/e* 404, 259. Anal. ($C_{20}H_{24}N_2O_7$) C, H, N.

1-Hydroxy-7-methoxymitosene Chloroacetate (28). A suspension of potassium carbonate (2 g) in ethyl acetate (2 mL) was treated with chloracetyl chloride (0.7 mL, 8.8 mmol) and stirred for a few minutes before adding a solution of **7** (16 mg, 0.05 mmol) in ethyl acetate (7 mL). The mixture was then stirred under nitrogen at room temperature for 12 h and diluted with water and ethyl acetate, and the organic phase was washed several times with water. After drying over magnesium sulfate and evaporation of solvent, the residue was purified by LPC, eluting with methylene chloride–acetone (9:1) to give **28** (9.7 mg, 48.9%) as a bright orange solid: mp 135 °C dec; NMR ($CDCl_3$) δ 6.36 (d of d, 1 H), 5.29 (s, 2 H), 4.76 (broad, 2 H), 4.32 (t, 2 H), 4.10 (s, 2 H), 4.08 (s, 3 H), 2.75 (m, 2 H), 1.97 (s, 3 H); MS, *m/e* 396, 398 (3:1), 259. Anal. ($C_{17}H_{17}N_2O_7Cl$) C, H, N.

1-Hydroxy-7-methoxymitosene Nicotinate (29). A solution of **7** (33.4 mg, 0.10 mmol), methylene chloride (6 mL), and dry pyridine (1 mL) was treated with nicotinyl chloride hydrochloride (150 mg, 1.09 mmol) and heated at reflux under nitrogen for 6 h. After the solution was cooled, water (0.25 mL) was added and the mixture was stirred vigorously for 2 min to hydrolyze excess acid chloride. It was then dried over magnesium sulfate and evaporated to an oil that smelled of pyridine. This oil was repeatedly diluted with toluene and evaporated at reduced pressure until no pyridine odor could be detected. The solid was triturated with methylene chloride in order to dissolve the orange product, which was filtered from undissolved nicotinic acid byproduct. The methylene chloride solution was then purified by LPC, eluting with methylene chloride–acetone–methanol (78:20:2) to give **29** (29.0 mg, 64.5%) as a yellow solid: mp 175–178 °C dec; IR 3460, 3370, 1732, 1640 cm^{-1}; NMR ($CDCl_3$ + Me_2SO-d_6) δ 9.12 (s, 1 H), 8.79 (d of d, 1 H), 8.31 (d of d, 1 H), 7.46 (d of d, 1 H), 6.44 (d of d, 1 H), 5.98 (broad, 2 H), 5.20 (s, 2 H), 4.35 (t, 2 H), 3.99 (s, 3 H), 2.85 (m, 2 H), 1.89 (s, 3 H); MS *m/e* 364 ($M^+ - HOCONH_2$), 259, 78 ($C_5H_4N^+$). Anal. ($C_{21}H_{19}N_3O_7$) C, H, N.

9-(Chloromethyl)-2,3-dihydro-1-hydroxy-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione Methylcarbamate (34). A solution of **6** (36 mg), methylene chloride (3.5 mL), and acetic anhydride (2.5 mL) was cooled to 0 °C and bubbled with anhydrous hydrogen chloride for 5 min (saturation achieved). The mixture was stirred 10 min more and then flushed with nitrogen for 10 min before evaporation at reduced pressure. The residual solid was chromatographed immediately on silica gel, eluting with methylene chloride–acetone (9:1). Collection of a major yellow band gave **32** (24.2 mg, 82.5%) as a peach-colored solid upon evaporation from methylene chloride–hexane: mp 136–138 °C dec; IR 3510, 1637 cm^{-1}; NMR ($CDCl_3$) δ 5.34 (d of d, 1 H), 4.91 (s, 2 H), 4.57 (broad, 1 H), 4.31 (t, 2 H), 4.00 (s, 3 H), 2.65 (m, 2 H), 1.97 (s, 3 H); MS, *m/e* 295, 297 (3:1), 260. The amount of water present on the silica gel was critical in obtaining the product. Silica gel dried 12 h at 130 °C gave a faster eluting product, possibly **31**, which decomposed as it left the column. Silica gel direct from the container gave **32** and traces of **33**. Silica gel that had been moistened by suspension in methylene chloride and air drying gave **33**: mp 164–165 °C; IR 3220, 1640 cm^{-1}; NMR δ 5.27 (m, 1 H), 4.86 (d, 2 H), 4.27 (m, 2 H), 4.05 (s, 3 H), 2.65 (m, 2 H), 1.97 (s, 3 H); MS, *m/e* 277, 259.

Compound **32** (24 mg, 0.09 mmol) was dissolved in methylene chloride (4 mL) and treated with anhydrous potassium carbonate (750 mg) followed by methyl isocyanate (0.3 mL, 4.9 mmol). The reaction mixture was stirred under argon in a stoppered flask for 14 h at room temperature, and then the potassium carbonate was filtered and rinsed well with methylene chloride. The combined washings and filtrate were evaporated at reduced pressure, and the residue was chromatographed on oven-dried silica gel with

methylene chloride–acetone (95:5) to give **34** (21 mg, 72.7%) as a yellow solid: mp 174 °C dec; IR 3300, 1690, 1637 cm^{-1}; NMR ($CDCl_3$) δ 6.15 (d of d, 1 H), 4.86 (s, 2 H), 4.70 (broad, 1 H), 4.33 (d of d, 2 H), 4.07 (s, 3 H), 2.82 (m, 5 H), 1.98 (s, 3 H); MS, *m/e* 352, 354 (3:1), 259. Anal. ($C_{16}H_{17}N_2O_5Cl$) C, H, N.

2,3-Dihydro-9-(hydroxymethyl)-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione 1-Oxime Carbamate (35). A solution of 7-methoxymitosen-1-one[11] (25 mg, 0.08 mmol), pyridine (5 mL), methanol (5 mL), and methylene chloride (12 mL) was treated with hydroxylamine hydrochloride (100 mg, 1.44 mmol) and stirred for 4 h at room temperature. During that time the color changed from yellow to bright red. The reaction mixture was diluted with 20 mL of methylene chloride, extracted with water (2 × 50 mL), dried over magnesium sulfate, and concentrated. The resulting solution in pyridine was diluted with toluene and evaporated under a vacuum to give a maroon solid. Purification was achieved by chromatography on silica gel, after prior adsorption of the product on a small quantity of the gel, by eluting with methylene chloride–acetone–methanol (8:1:1). This procedure gave **35** (17.4 mg, 66.5% from 31) as a maroon solid: mp 200 °C dec; IR 3600–3520, 1690, 1655, 1640; NMR (Me_2SO-d_6 + $CDCl_3$) δ 6.17 (broad, 2 H), 5.11 (s, 2 H), 4.34 (m, 2 H), 3.94 (s, 3 H), 1.87 (s, 3 H); MS, *m/e* 333, 272.

Compound **35** was purified for elemental analysis by semipreparative scale HPLC as follows: A solution of **35** (5 mg) in methylene chloride–acetone (1:1, 6 mL) was chromatographed (1.0 mL per run) by eluting first with methylene chloride for 6 min and then introducing a 0–5% linear gradient of methanol over 20 min. The elution rate was 5 mL/min. The acetone eluted first, followed by a minor component. Next the major, red-colored component **35** eluted, followed closely by two minor components. Collection of center cuts from the major peak on each run gave **35** as a red-brown solid upon evaporation of solvent. A small sample was rechromatographed as above to give a single sharp peak on the recorder chart, indicating pure **35**, which was vacuum dried for 24 h. Anal. ($C_{15}H_{15}N_3O_6$) C, H, N.

7-(1-Aziridinyl)-1-hydroxymitosene Acetate (36). A solution of **6** (38 mg, 0.12 mmol) in dry methanol (50 mL) was treated with ethylenimine (2 mL) and stirred for 2.5 h at room temperature. The reaction mixture was then evaporated at reduced pressure and chromatographed on silica gel with methylene chloride–acetone (4:1). Collection of the major red band and evaporation of solvent gave **36** (26.8 mg, 68.7%) as a burgundy-colored solid: mp 204–205 °C dec; IR 3450–3200, 1730, 1640; NMR ($CDCl_3$) δ 6.30 (d of d, 1 H), 5.28 (s, 2 H), 4.86 (broad, 2 H), 4.27 (t, 2 H), 2.80 (s, 4 H), 2.07 (s, 6 H); MS, *m/e* 373, 270. Further purification was possible by recrystallization from ethanol: mp 208–210 °C dec. Anal. ($C_{16}H_{19}N_3O_6$) C, H, N.

A second, smaller red band that eluted from the column after **36** gave about 5 mg of a burgundy-colored solid that appeared by its NMR spectrum to be 7-(1-aziridinyl)-1-hydroxymitosene: NMR ($CDCl_3$ + Me_2SO-d_6) δ 6.31 (m, 1 H), 5.90 (broad, 2 H), 5.19 (d, 2 H), 4.22 (t, 2 H), 297 (s, H_2O), 2.26 (s, 4 H), 1.99 (s, 3 H).

7-(1-Aziridinyl)-1-hydroxymitosene Carbamate (37). Compound **24** (33 mg) was dissolved in DMF (5 mL, dried over potassium hydroxide and distilled) and treated with ethylenimine (1 mL). The solution changed from yellow to deep red in 20 min but the mixure was allowed to stir a total of 3 h at room temperature before evaporation of solvent under vacuum. The red solid residue was suspended in chloroform (25 mL) and stirred with silica gel (5 g) until all of the solid was adsorbed on the gel (about 45 min). Solvent was removed at reduced pressure and the adsorbed residue was purified by chromatography on silica gel, eluting with chloroform–methanol–acetonitrile (94:3:3). Collection of the major red band and evaporation of solvent at reduced pressure gave **37** (30 mg, 88.2%) as a bright red solid: mp 200–205 °C dec; IR 3440, 3320, 3200, 2920, 1690, 1660, 1640 cm^{-1}; NMR (Me_2SO-d_6) δ 6.66 (broad, 2 H), 6.39 (broad, 2 H) 5.98 (m, 1 H), 5.03 (d, 2 H), 4.21 (m, 2 H), 2.85 (m, 2 H), 2.24 (s, 4 H), 1.94 (s, 3 H); MS, *m/e* 374, 270.

Repeated attempts to obtain an elemental analysis gave results inconsistent with the molecular formula despite the overwhelming spectral evidence supporting the designated structure. Hence, **37** was purified by semipreparative scale HPLC as follows: A solution of **37** (15 mg) in DMF–methylene chloride (3:1, 5 mL) was chromatographed (ca. 0.5 mL per run) by eluting first with methylene chloride for 3 min and then introducing a 0–5% linear gradient of methanol over 20 min. Elution rate was 5 mL/min. Some nonpolar material eluted first, followed by DMF. Next to elute were the major pink fraction and two minor components. Center cuts of the major component were collected for each run and combined. A portion (1.0 mL) was then injected and eluted as above to give a single sharp peak on the recorder chart, indicating pure **37**. Evaporation of solvent under a vacuum gave **37** as a red solid. The solid was ground to a fine powder and dried under a vacuum at room temperature for 48 h before submission for elemental analysis. The results were several percent high for C and H and low for N, indicating trapped solvent. A high-resolution mass spectrum of the Me_4Si derivative failed to give a strong molecular ion despite its presence in the low-resolution spectrum; however, M^+ – HNCO was within 5 mmu of the theoretical value of 475.2032 ($C_{22}H_{33}N_3O_5Si_2$).

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Supplementary Material Available: Full screening data for compounds submitted to the P388 leukemia assay (Table I) (2 pages). Ordering information is given on any current masthead page.