Mitomycin Antibiotics. Synthesis of 1-Substituted 7-Methoxymitosenes[1]

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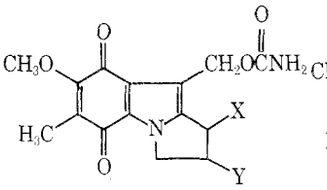
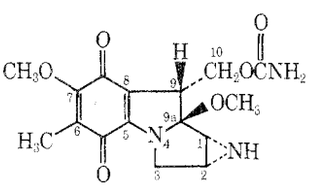
A route to 1-substituted 7-methoxymitosenes was developed starting from 2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indol-1-one. It involved conversion of the 1-oxo function into an acetamido or acetoxy group followed by 9-formylation, quinone-ring elaboration, and conversion of the formyl group into a hydroxymethyl carbamate. Mitosenes with 1-acetamido and 1-acetoxy substituents thus were prepared and the 1-acetoxy compound was further transformed into 1-hydroxy and 1-oxo analogs. Elaboration of the quinone ring involved the shortened sequence of nitration, reduction, and Fremy's salt oxidation which had not yet been applied to mitosenes. Preparation of the starting pyrroloindol-1-one was shortened two steps by making a simplification in the Reissert indole synthesis.

In 1964 the synthesis of 7-methoxymitosene (**1**) was reported by Allen and coworkers.[2] This compound has all of the structural features of the naturally occurring mitomycins (*e.g.*, **3**) except for the aziridine ring and elements of



1, X = Y = H
2, X = OH; Y = NHAc



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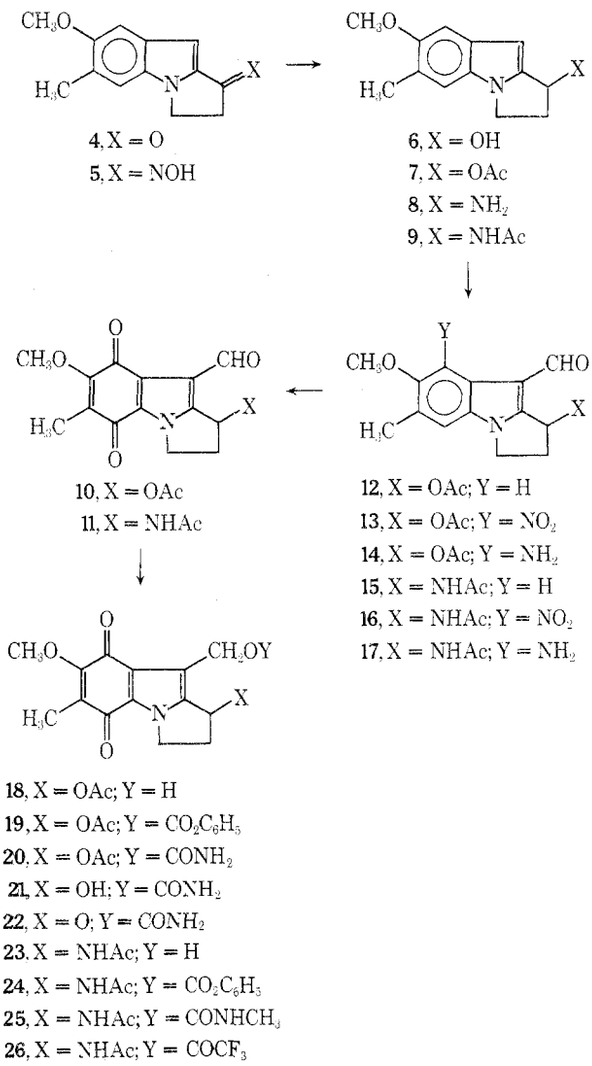
methanol at the 9 and 9a positions. It lacks the antitumor and some of the antibacterial activity of the mitomycins, although it is active against *Staphylococcus aureus* infections in mice.[2] Subsequent synthesis of mitomycin analogs have been directed toward simpler compounds such as indoloquinone carbamates[3,4] and benzoquinone carbamates.[5-7] Although certain of these analogs showed interesting biological activities, none was even close to the mitomycins in potency and spectrum of activity.

These results prompted the design and synthesis of analogs more closely related in structure to the mitomycins. In this article we describe the synthesis of 1-substituted mitosenes. These compounds are close analogs of the apomitomycins such as **2**, which have been reported to have both antitumor and antibacterial activities.[8,9] They appear to afford the possibility of bifunctional alkylation at positions 1 and 10, especially when there is a leaving group at the 1 position. The functional groups we wished to have at the 1 position included hydroxy, acetoxy, and acetamido since all of these groups occur in apomitomycins with antitumor activity.[8,9]

For the synthesis of 1-substituted mitosenes, the earlier synthesis of 7-methoxymitosene[2] was obviously important, since it showed how to elaborate the quinone and carbamate functions on the pyrrolo[1,2-*a*]indole nucleus substituted with a 9-hydroxymethyl group. We anticipated that certain simplifications could be made in these elaborations, especially by utilizing the nitration route (**15** → **11**) for preparation of the qunione ring. This route had been developed for simpler indole derivatives,[10] but it had not been applied to pyrroloindoles.

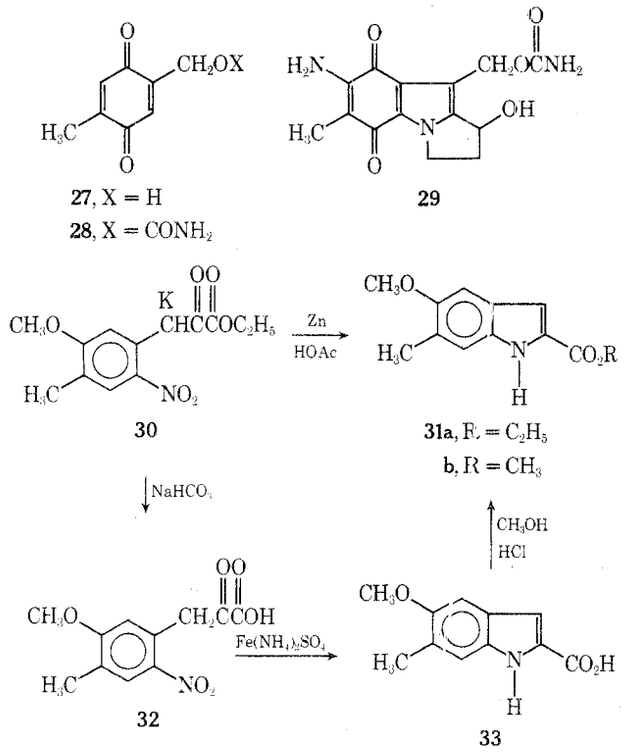
The introduction of a 9-hydroxymethyl group into a 1-substituted pyrroloindole had been investigated previously,[11] and it appeared that the same method would succeed in the preparation of 1-substituted mitosenes provided that a proper sequence of operations was devised. The sequence of operations which proved to be successful was based upon the known 1-oxopyrroloindole **4**[2] and it involved conversion of **4** into the corresponding 1-acetoxy derivative **7** or 1-acetamido derivative **8**, followed by introduction of the 9-formyl group which stabilized the molecule through subsequent steps in elaboration of the quinone ring. The formyl group was finally converted into the 9-hydroxymethyl carbamate and further transformations at the 1 position were completed.



4, X = O
5, X = NOH
6, X = OH
7, X = OAc
8, X = NH_2
9, X = NHAc
10, X = OAc
11, X = NHAc
12, X = OAc; Y = H
13, X = OAc; Y = NO_2
14, X = OAc; Y = NH_2
15, X = NHAc; Y = H
16, X = NHAc; Y = NO_2
17, X = NHAc; Y = NH_2
18, X = OAc; Y = H
19, X = OAc; Y = $CO_2C_6H_5$
20, X = OAc; Y = $CONH_2$
21, X = OH; Y = $CONH_2$
22, X = O; Y = $CONH_2$
23, X = NHAc; Y = H
24, X = NHAc; Y = $CO_2C_6H_5$
25, X = NHAc; Y = $CONHCH_3$
26, X = NHAc; Y = $COCF_3$

1-Oxopyrroloindole **4** had been prepared previously by a nine-step sequence starting from 3,5-xyenol,[2] but we found

that this sequence could be shortened by two steps. In the earlier route, the potassium salt **30** formed by condensing 2,5-dimethyl-4-nitroanisole with ethyl oxalate and potassium *tert*-butoxide was hydrolyzed to nitrophenylpyruvic acid derivative **32.** This derivative was reductively cyclized



with ferrous ammonium sulfate and the resulting indole-2-carboxylic acid **33** was esterified to give **31b.** The shortened sequence involved treatment of potassium salt **30** with zinc in acetic acid to give indole-2-carboxylic acid ester **31a** directly. This sequence gave a somewhat lower overall yield (35%) from 2,5-dimethyl-4-nitroanisole than longer one (42%) but it was much faster and easier to perform on a large scale.

The 1-oxopyrroloindole **4,** prepared by either of these two routes, was used for the synthesis of 1-acetamido-7-methoxymitosene (**25**), 1-acetoxy-7-methoxymitosene (**20**), and compounds derived from them. For the 1-acetamidomitosene compounds, **4** was converted to the corresponding oxime **5,** which was reduced catalytically to 1-aminopyrroloindole **8.** This amine was unstable and it could not be isolated; however, addition of acetic anhydride and triethylamine to the reduction mixture afforded the corresponding 1-acetamido compound **9** in fair yield. After formylation of **9** under Vilsmeier–Haack conditions, the resulting 9-formyl derivative **15** was nitrated to give **16,** and this compound was reduced to 8-amino derivative **17** with iron and acetic acid. Treatment of **17** with potassium nitrosodisulfonate (Fremy's salt) then gave quinone **11.** Conversion of the formyl group of **11** into the corresponding hydroxymethyl group of compound **23** was accomplished by Allen's procedure,[2] which involves sodium borohydride reduction followed by ferric chloride oxidation to regenerate the quinone. Formation of a carbamate derivative of **23** proved to be surprisingly difficult and it contrasted the easy formation of a carbamate (**20**) from the 1-acetoxy analog. Phenylcarbonate **24** was prepared readily from **23** but it gave only **23** and phenol upon treatment with anhydrous ammonia.

An alternative route to the carbamate synthesis, involving treatment of the hydroxymethyl compound **23** with sodium cyanate in trifluoroacetic acid,[12] was attempted. This method gave only trifluoroacetate derivative **26** when applied to **24.** However, it gave an 87% yield of carbamate **28** when applied to hydroxymethylbenzoquinone (**27**).

Successful synthesis of a carbamate derivative was achieved finally by treatment of **23** with methyl isocyanate and triethylamine[5] to give methylcarbamate **25.**

Since the synthesis of 1-acetoxy-7-methoxymitosene (**20**) from **4** was described in a previous communication[1] it will not be recapitulated extensively here. The synthetic procedures, which involve a route through compounds **6, 7, 12, 13, 14, 10, 18,** and **19,** are described in the Experimental Section. It should be mentioned that the first three steps in this route were based upon previous work in the related 7-benzyloxypyrroloindoles,[11] but the 7-methoxy-6-methylpyrroloindoles **6** and **7** proved to be very unstable toward acid and special conditions had to be developed for them.

As previously reported,[1] selective hydrolysis of **20** gave 1-hydroxy-7-methoxymitosene (**21**), which was converted into 7-methoxymitosen-1-one (**22**) by manganese dioxide. Thus the first synthesis of a major degradation product of the mitomycins (desammonoapomitomycin A)[13] was accomplished.

Biological activities of the 1-substituted mitosenes described above will be reported elsewhere.

Experimental Section

Melting points were determined on a Mel-Temp apparatus and are uncorrected. Infrared spectra were recorded on a Beckman IR-33 spectrophotometer. Uv spectra were determined in methanol on a Varian Cary Model 17 recording spectrophotometer. Nmr spectra were determined on a Japan Electron Optics Laboratory NMH-6011 spectrometer in Me_2SO-d_6 with tetramethylsilane as an internal standard. The nmr spectrum of each compound reported below agreed with its structure. Only significant nmr peaks are given below since most of the spectra were routine. Solutions were dried using $MgSO_4$ and concentrated under reduced pressure on a rotary evaporator. Elemental analyses were determined by the Microanalytical Laboratory, Department of Chemistry, Purdue University.

Ethyl 5-Methoxy-6-methylindole-2-carboxylate (31a). A stirred mixture of potassium salt **30** (prepared in the usual way from 100 g of 2,5-dimethyl-4-nitroanisole)[2] and 1 l. of glacial acetic acid was treated with 250 g of zinc dust added at a rate to maintain the temperature near 45°. After this addition was complete the mixture was stirred 3 hr and filtered, and the solids were washed with acetic acid. The combined filtrate and washes were diluted with 3 l. of water to give an oil which was separated and concentrated under reduced pressure. The dark solid residue was washed thoroughly with methanol to afford 45.6 g (35% from 2,5-dimethyl-4-nitroanisole) of nearly colorless solid which after recrystallization from ethanol had mp 165–166°; ir 2.96 (NH), 5.88 μ (CO_2Et); uv 294 nm (18,400).

Anal. Calcd for $C_{13}H_{15}NO_3$: C, 66.94; H, 6.48; N, 6.01. Found: C, 66.71; H, 6.46; N, 6.12.

2,3-Dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indol-1-one Oxime (5). A mixture of 11.5 g of **4**,[2] 5.5 g of hydroxylamine hydrochloride, 100 ml of ethanol, and 100 ml of pyridine was heated on a steam bath for 90 min, cooled, and concentrated under reduced pressure. Crystallization of the residue from methanol gave 12.2 g (100%) of white crystals: mp 220–222°; uv 322 nm (25,000).

Anal. Calcd for $C_{13}H_{14}N_2O_2$: C, 67.81; H, 6.13; N, 12.17. Found: C, 67.58; H, 5.83; N, 11.97.

1-Acetamido-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole (9). A mixture of 6.25 g of **5,** 6.0 g of 5% palladium-on-carbon, 200 ml of acetone, 10 ml of acetic anhydride, and 25 ml of triethylamine was shaken with hydrogen at an initial pressure of 50 psi for 24 hr. It was filtered and the catalyst was extracted in a sohxlet apparatus for 6 hr. Concentration of the combined filtrate and extract gave an oil which was crystallized from ether. This procedure gave 4.02 g (57%) of white solid: mp 199–201°; ir (KBr) 6.72 μ ($NHCOCH_3$); uv 217 (40,000), 277 (11,000), 296 (8400), 307 nm (3000).

Anal. Calcd for $C_{15}H_{18}N_2O_2$: C, 69.74; H, 7.03; N, 10.85. Found: C, 69.59; H, 6.89; N, 10.77.

1-Acetamido-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyr-

rolo[1,2-*a*]indole-9-carboxaldehyde (15). A solution of 4.14 g of **9** in 70 ml of *N,N*-dimethylformamide was added during 1 hr to an ice-cooled, stirred mixture of 2.0 ml of phosphorus oxychloride and 20 ml of *N,N*-dimethylformamide. After another hour the resulting mixture was poured into 500 ml of saturated sodium acetate solution. The yellow solid which formed overnight was collected and recrystallized from ethanol to give 4.18 g (92%) of yellow crystals: mp 285–287°; ir (KBr) 6.1 (CHO), 6.60 μ ($NHCOCH_3$); uv 252 (19,000), 280 (14,000), 308 nm (13,000).

Anal. Calcd for $C_{16}H_{18}N_2O_3$: C, 67.12; H, 6.34; N, 9.78. Found: C, 66.89; H, 6.32; N, 9.59.

1-Acetamido-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (16). A mixture of 4.21 g of **15** and 45 ml of glacial acetic acid was stirred at 10° and treated during 30 min with 4.50 ml of 90% nitric acid. The mixture was stirred 1 hr and treated with 200 ml of water, which caused crystallization of product. This product was washed with ethanol and dried to give 3.90 g (80%) of yellow solid: mp 277–279° dec; ir (KBr) 6.1 (CHO), 6.5, 7.3 μ (NO_2).

Anal. Calcd for $C_{16}H_{17}N_3O_5$: C, 58.00; H, 5.17; N, 12.68. Found: C, 57.81; H, 5.44; N, 12.71.

1-Acetamido-2,3-dihydro-7-methoxy-6-methyl-5,8-dioxo-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (11). A solution of 3.9 g of **16** in 300 ml of 50% aqueous acetic acid was stirred with 7.0 g of iron filings for 24 hr at room temperature, diluted with 1 l. of water, and extracted with methylene chloride. The organic layer was extraced with 6 *N* hydrochloric acid and this extract was neutralized with sodium carbonate, whereupon 2.80 g of amine **17** precipitated. This amine showed only one spot on tlc and had the appropriate ir spectrum (2.9, 5.95, 6.2 μ), but it was too unstable for purification for analysis. It was converted directly to **11** as follows.

A solution of 10.0 g of potassium nitrosodisulfonate in 500 ml of water and 240 ml of 0.167 *M* potassium dihydrogen phosphate was treated with 2.8 g of **17** in 500 ml of acetone. The mixture was stirred 12 hr, diluted with water, and extracted with methylene chloride. This extract was washed with water, dried, and concentrated to give a red solid which was washed with hot methanol. This procedure gave 1.4 g (38% from **16**) of orange solid which decomposed without melting above 280°: ir (KBr) 6.0 μ (CHO); uv 242 (13,400), 270 (14,600), 297 (11,800), 333 nm (5700).

Anal. Calcd for $C_{16}H_{16}N_2O_5$: C, 60.76; H, 5.10; N, 8.86. Found: C, 60.56; H, 5.29; N, 8.71.

1-Acetamido-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (23). A solution of 580 mg of **11** in 50 ml of tetrahydrofuran and 10 ml of ethanol, under nitrogen, was treated with 780 mg of sodium borohydride, stirred 40 min, and then treated with 10 ml of acetone. After 10 min the mixture was treated with 7.0 ml of 1 *N* ferric chloride in 0.1 *N* hydrochloric acid. Water and methylene chloride were added and the organic layer was washed with water, dried, and concentrated. The orange solid residue was washed with ether to give 490 mg (86%) of product: mp 241° dec; ir (KBr) 3.1 (OH), 6.0, 6.1 μ (C=O); uv 228 (17,500), 285 (13,600), 350 (3400), 445 nm (950).

Anal. Calcd for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70. Found: C, 60.28; H, 5.95.

1-Acetamido-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione Phenylcarbamate (24). A solution of 200 mg of **23** in 20 ml of pyridine was treated at 0° with 186 mg of phenyl chloroformate. The mixture was stirred 4 hr at room temperature and poured into water and methylene chloride. The organic layer was washed successively with 3 *N* hydrochloric acid, 5% sodium bicarbonate, and water, dried, and concentrated. Two recrystallizations of the resulting solid from methylene chloride–hexane gave 138 mg (47%) of orange crystals: mp 199° dec; ir (KBr) 5.70 μ ($OCO_2C_6H_5$).

Anal. Calcd for $C_{23}H_{22}N_2O_7$: C, 63.01; H, 5.06. Found: C, 63.27; H, 5.46.

1-Acetamido-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione Methylcarbamate (25). A mixture of 76 mg of **23**, 20 ml of tetrahydrofuran, 20 ml of methyl isocyanate, and 0.25 ml of triethylamine was heated at reflux temperature for 6 hr and concentrated, and the residual solid was purified by chromatography on magnesia–silica gel with 1:1 chloroform–acetone as solvent. Concentration of the orange band gave a golden solid which after recrystallization from ethanol weighed 42 mg (47%) and had mp 240° dec: ir (KBr) 5.90 μ (OCONH).

Anal. Calcd for $C_{18}H_{21}N_3O_6$: C, 57.59; H, 5.64; N, 11.19. Found: C, 57.27; H, 5.45; N, 11.10.

1-Acetamido-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione Trifluoroacetate (26). A mixture of 50 mg of **23**, 1.2 g of trifluoroacetic acid, 750 mg of sodium cyanate, and 20 ml of methylene chloride was stirred 4.5 hr and treated with water. The organic layer was washed with 10% sodium bicarbonate, dried, and concentrated. The residual solid was washed with alcohol and then recrystallized from methylene chloride–hexane to give 15 mg (27%) of orange crystals: mp 198° dec; ir 5.6 μ ($OCOCF_3$).

Anal. Calcd for $C_{18}H_{17}F_3N_2O_6$: C, 52.18; H, 4.15; N, 6.76. Found: C, 52.30; H, 4.24; N, 6.96.

2-Hydroxymethyl-5-methyl-1,4-benzoquinone Carbamate (28). A mixture of 310 mg of **27**, 200 mg of sodium cyanate, and 0.5 ml of trifluoroacetic acid in 20 ml of methylene chloride was stirred for 16 hr and then concentrated under reduced pressure. The residue was washed with ether and with water and dried in a vacuum desiccator to give 358 mg (84%) of yellow solid: mp 119–122° dec; ir (KBr) 2.9 (NH_2), 5.9 ($OCONH_2$), 6.1 μ (C=O).

Anal. Calcd for $C_9H_9NO_4$: C, 55.39; H, 4.65; N, 7.18. Found: C, 55.17; H, 4.81; N, 7.40.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole (7). To a suspension of 0.5 g of **4** in 70 ml of ethanol was added 0.36 g of sodium borohydride. The mixture was stirred 4 hr and concentrated under reduced pressure, and the residue was treated with ether and water. The ether layer was washed with water, dried, and concentrated to give 0.48 g of crude alcohol **6**, which was used directly in the next step.

A stirred solution of 0.45 g of crude **6** in 8.9 ml of pyridine was treated with 8.9 ml of acetic anhydride. After 16 hr the mixture was diluted with water and extracted with ether. This extract was washed consecutively with 3 *N* hydrochloric acid, 5% sodium bicarbonate, and water. It was dried and concentrated to a solid residue which was crystallized from petroleum ether to give 0.43 g (72%) of white crystals: mp 134–135°; ir (KBr), 5.75 μ; uv 216 (44,000), 279 (11,000), 300 (8400), 312 nm (3100).

Anal. Calcd for $C_{15}H_{17}NO_3$: C, 69.49; H, 6.55; N, 5.40. Found: C, 69.24; H, 6.56; N, 5.32.

In subsequent experiments, yields up to 90% were obtained for **7**.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (12). A solution of 0.42 g of **7** in 10 ml of *N,N*-dimethylformamide was added during 1 hr to a stirred, ice-cooled solution of 0.25 g of phosphorus oxychloride in 2 ml of *N,N*-dimethylformamide. After an additional hour the resulting solution was added dropwise to a stirred solution of saturated aqueous sodium acetate at −10°. The mixture was stirred 2 hr at 0° and filtered, and the crude solid product was washed with water and recrystallized from isopropyl ether. This procedure gave 0.33 g (77%) of light tan needles: mp 170–172°; ir (KBr) 5.75 ($OCOCH_3$), 6.0 μ (CHO); nmr δ 9.9 (s, CHO).

Anal. Calcd for $C_{16}H_{17}NO_4$: C, 66.89; H, 5.92; N, 4.87. Found: C, 66.75; H, 5.92; N, 4.80.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-8-nitro-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (13). A stirred solution of 0.50 g of **12** in 10 ml of glacial acetic acid at 20° was treated with 0.50 ml of 90% nitric acid. After 30 min the mixture was diluted with water and filtered. Recrystallization of the crude solid from ethanol gave 0.40 g (70%) of yellow solid: mp 175–176°; ir 5.75 ($OCOCH_3$), 6.0 (CHO), 6.5, 7.3 μ (NO_2); uv 216 (36,000), 248 (18,500), 304 nm (12,300).

Anal. Calcd for $C_{16}H_{16}N_2O_6$: C, 57.83; H, 4.82; N, 8.43. Found: C, 57.88; H, 4.79; N, 8.31.

1-Acetoxy-2,3-dihydro-7-methoxy-6-methyl-5,8-dioxo-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (10). A solution of 0.80 g of **13** in 65 ml of 50% aqueous acetic acid was heated at 80° with 1.40 g of iron filings. The mixture was stirred for 2 hr, diluted with water, and extracted with methylene chloride. This extract was shaken with 6 *N* hydrochloric acid and the acidic layer was neutralized with sodium carbonate. The crude amine **14** which separated (0.80 g) was used directly in the next step.

To a solution of 2.30 g of potassium nitrosodisulfonate in 120 ml of water and 60 ml of 0.167 *M* potassium dihydrogen phosphate was added a solution of 0.80 g of crude **14** in 120 ml of acetone. After 16 hr the mixture was diluted with water and extracted with methylene chloride. This extract was washed with water, dried, and concentrated to an orange solid, which was purified by chromatography on silica gel with chloroform as solvent. Recrystallization of the purified product from ethanol gave 0.30 g (39%) of orange crystals: mp 126–127°; ir 5.75 ($OCOCH_3$), 6.0 μ (CHO); uv 243 (13,500), 272 (14,700), 297 (11,800), 334 nm (5800).

Anal. Calcd for $C_{16}H_{15}NO_6$: C, 60.60; H, 4.73; N, 4.41. Found: C, 60.82; H, 4.82; N, 4.41.

1-Acetoxy-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione (18). A solution of 100 mg of **10** in 10 ml of ethanol and 5 ml of tetrahydrofuran, under nitrogen, was treated at 0° with 120 mg of sodium borohydride. After 15 min 1 ml of acetone was added and stirring was continued 5 min. The solution was treated with 1 ml of 1 *N* ferric chloride in 0.1 *N* hydrochloric acid and then water and methylene chloride were added. The organic layer was washed with water, dried, and concentrated. Chromatography of the concentrate on silica gel with chloroform as solvent gave the product in a single orange band, which afforded upon concentration 60 mg (60%) of orange solid. Recrystallization from methylene chloride–hexane gave crystals: mp 123–124°; ir (film) 2.90 (OH), 5.75 μ ($OCOCH_3$); uv 230 (17,000), 286 (13,400), 350 (3330), 450 nm (1000).

Anal. Calcd for $C_{16}H_{17}NO_6$: C, 60.18; H, 5.37; N, 4.39. Found: C, 59.93; H, 5.62; N, 4.59.

1-Acetoxy-2,3-dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione Carbamate (1-Acetoxy-7-methoxymitosene, 20). A solution of 0.51 g of **18** in 50 ml of pyridine was treated at 0° with 2 ml of phenyl chloroformate. The mixture was stirred overnight at room temperature, diluted with water, and extracted with methylene chloride. This extract was washed consecutively with 3 *N* hydrochloric acid, 5% sodium bicarbonate, and water, dried, and concentrated. The residue was chromatographed on silica gel with benzene as solvent, which eluted some diphenyl carbonate. The desired phenylcarbonate **19** was obtained by eluting with chloroform in 0.50-g (71%) yield. It was used directly in the next step.

A solution of 175 mg of **19** in 15 ml of methylene chloride was cooled in a Dry Ice–acetone bath and anhydrous ammonia was bubbled through it for 30 min. It was then stirred at room temperature for 2 hr, warmed to expel excess ammonia, washed with water, dried, and concentrated. Crystallization of the residual solid from ethanol gave 110 mg (75%) of orange cyrstals: mp 204–205° dec; ir (Nujol) 3.02–3.24 (NH_2), 5.75 ($OCOCH_3$), 6.06 μ ($OCONH_2$); nmr ($CDCl_3$) s 4.6–5.0 (m, 2, $OCONH_2$).

Anal. Calcd for $C_{17}H_{18}N_2O_7$: C, 56.35; H, 5.00; N, 7.73. Found: C, 56.21; H, 5.23; N, 7.35.

2,3-Dihydro-1-hydroxy-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,7-dione Carbamate (1-Hydroxy-7-methoxymitosene, 21). A solution of 40 mg of **20** in 60 ml of 0.25 *N* ammonium hydroxide in methanol was stirred 20 hr at room temperature and concentrated, and the residual solid was purified by chromatography on silica gel with 4:1 chloroform–acetone as solvent. Concentration of the main orange band gave 31 mg (87%) of orange crystals: mp 195–196°; ir (KBr) 2.95 (OH), 3.00–3.20 (NH_2), 5.90 μ ($OCONH_2$).

Anal. Calcd for $C_{15}H_{16}N_2O_6$: C, 56.25; H, 5.04; N, 8.75. Found: C, 56.33; H, 5.15; N, 8.91.

2,3-Dihydro-9-hydroxymethyl-7-methoxy-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-1,5,8-trione Carbamate (7-Methoxymitosen-1-one, 22). A solution of 20 mg of **21** in 10 ml of methylene chloride was stirred 24 hr at room temperature with 200 mg of activated manganese dioxide. It was filtered and the solids were washed thoroughly with methylene chloride. The combined filtrate and wash was concentrated and the residue was purified by thick layer chromatography on silica gel plates with 1:1 chloroform–acetone as solvent. The orange solid product was identical in infrared spectrum, mass spectrum, and parallel and overspot thin-layer chromatography with an authentic sample (desammonoapomitomycin A).[13]

7-Amino-2,3-dihydro-1-hydroxy-9-hydroxymethyl-6-methyl-1*H*-pyrrolo[1,2-*a*]indole-5,8-dione Carbamate (7-Amino-1-hydroxymitosene, 29). Anhydrous ammonia was bubbled into a solution of 30 mg of **21** in 200 ml of methanol at 0° for 1 hr. The mixture was kept 30 hr at room temperature and concentrated, and the residue was purified by chromatography on magnesia–silica gel with 4:1 chloroform–acetone as solvent. The second band (violet colored) gave upon concentration 25 mg (87%) of purple solid which had after recrystallization from acetonitrile: mp 220–225° dec; ir (KBr) 2.90 (OH), 3.00–3.20 (NH_2), 5.90 μ ($OCONH_2$).

Anal. Calcd for $C_{14}H_{15}N_3O_5$: C, 55.08; H, 4.95; N, 13.76. Found: C, 54.81; H, 4.38; N, 13.74.

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Registry No.—**4,** 3139-14-8; **5,** 52674-12-1; **6,** 3188-36-1; **7,** 40863-71-6; **9,** 52718-88-4; **10,** 40863-75-0; **11,** 52718-89-5; **12,** 40863-72-7; **13,** 40863-73-8; **14,** 40863-74-9; **15,** 52674-13-2; **16,** 52674-14-3; **17,** 52674-15-4; **18,** 40863-76-1; **19,** 40863-77-2; **20,** 40863-78-3; **21,** 40863-79-4; **22,** 40863-80-7; **23,** 52718-90-8; **24,** 52718-91-9; **25,** 52718-92-0; **26,** 52718-93-1; **27,** 40870-52-8; **28,** 38439-89-3; **29,** 52748-18-2; **30,** 52674-16-5; **31a,** 52674-17-6.

References and Notes

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