Mitomycin Antibiotics. Synthesis of 7-Methoxy-1-(*N*-pyrrolidino)mitosene and Its Methiodide

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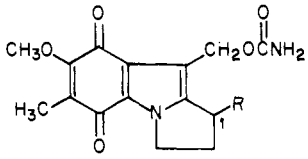
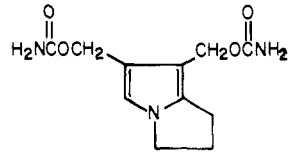
7-Methoxy-1-(*N*-pyrrolidino)mitosene and its methiodide were synthesized. The latter compound was a potential bifunctional alkylating agent because of its two good leaving groups appropriately situated with respect to the indoloquinone chromophore. However, it was inactive in bacteriophage induction and P388 murine leukemia assays. Both compounds showed antibacterial activity in culture, and the former compound was very weakly active in inducing lysogenic bacteriophage.

In the course of our studies on the synthesis of mitosene analogues of the mitomycins, we prepared a compound, 1-acetoxy-7-methoxymitosene (**1**),[2] which was active against



1, R = OAc
2, R = OH
3, R = NHAc



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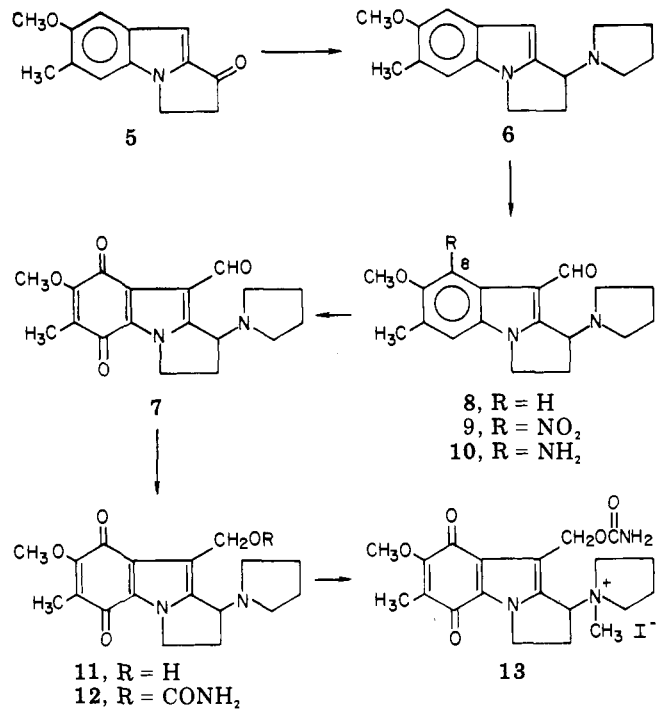
P388 leukemia in mice.[3] Since the corresponding 1-hydroxy analogue **2** and the 1-acetamido analogue **3** were inactive in this assay,[3] we reasoned that the presence of a good leaving group at position 1 might be important for antitumor activity. A good leaving group at position 1 plus the carbamate functionality of the mitosene would provide the basis for bifunctional alkylation and cross-linking of DNA, which is considered to be the lethal event for cancer cells.[4] Although this leaving-group hypothesis needs to be confirmed, it provides a starting point for the design of new mitosene analogues. Furthermore, it should be noted that diesters of 2,3-dihydro-6,7-bis(hydroxymethyl)-1*H*-pyrrolizine (e.g., **4**), which structurally resemble reduced 1-substituted mitosenes, exhibit significant antitumor activity that can be related to bifunctional alkylation.[5]

Since we had observed previously that the antibacterial activity of the 1-acetoxy compound **1** was diminished in the presence of serum,[6] we thought that the acetoxy group probably was undergoing partial enzymic hydrolysis. Therefore, it seemed desirable to prepare analogues of **1** which had good leaving groups at position 1 but which were stable to hydrolysis. Because the quaternary ammonium substituent appeared to fulfill these requirements, our goal became the synthesis of 7-methoxy-1-(*N*-pyrrolidino)-mitosene (**12**) and its conversion into the quaternary salt **13** with methyl iodide. Another reason for preparing quaternary ammonium compounds was that we were interested in the total synthesis of 7-methoxy-1,2-(*N*-methylaziridino)mitosene, and it appeared that Hofmann elimination at some stage in the synthetic pathway would give a 1,2-ene intermediate that might be converted into an aziridinomitosene.

The starting material for this synthesis was pyrrolo-[1,2-*a*]indol-1-one **5**.[2,7] It was converted into the corresponding 1-*N*-pyrrolidino derivative **6** in high yield by reductive amination in the presence of sodium cyanoborohydride. An alternative route involving formation of the pyrrolidine enamine followed by catalytic hydrogenation gave a lower yield of **6**. Vilsmeier–Haack formylation of **6** as the free base was unsatisfactory. However, when the monomaleate salt of **6** was used, a nearly quantitative yield of the 9-formyl derivative **8** was obtained. Nitration



5 **6**

7

8, R = H
9, R = NO_2
10, R = NH_2

11, R = H
12, R = $CONH_2$

13

of **8** readily gave a derivative **9**, which is considered to be an 8-nitro compound by analogy to the product formed when other pyrrolo[1,2-*a*]indole-9-carboxaldehydes and related indoles are nitrated.[8,9] Reduction of **9** under the usual conditions involving iron in acetic acid gave very poor yields of the corresponding amino derivative **10**, but catalytic hydrogenation of **9** produced **10** in good yields. No reduction of the carboxaldehyde group was observed. Fremy's salt oxidation of **10** to the quinone **7** was not satisfactory when it was conducted in pH 4.3 potassium dihydrogen phosphate solution. However, it went very well in pH 6.8 phosphate buffer solution. Conversion of quinone-9-carboxaldehyde **7** into the desired 9-hydroxymethyl carbamate **12** was accomplished by the usual sequence involving sodium borohydride reduction, ferric chloride oxidation, phenyl carbonate ester formation, and displacement of phenoxide by ammonia. The methiodide (**13**) of **12** was prepared readily by methyl iodide in tetrahydrofuran.

Biological Activity. Both 7-methoxy-1-(*N*-pyrrolidino)mitosene (**12**) and its methiodide **13** inhibit the growth of *Bacillus subtilis* in culture according to the disk plate assay (Table I). These compounds are much less potent than mitomycin C. However, **12** is approximately as potent as 1-acetoxymitosene (**1**), a compound which has significant antitumor activity. Compound **13** was active at the highest test dose, but it showed only a trace of inhibition at the next lowest doses. To be considered active in the λ-phage induction assay at a given concentration,

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Table I. Antibacterial and Phage Induction Activities[a]

Compd	Inhibn of *B. subtilis* at pH 8, zone in mm, concn in 10-mm disk in μg/mL[b]							Induction of λ phage of *E. coli*, ratio of plaques in treated culture to those in control, dose in μg/mL[c]					
	50	16.6	12.5	5.5	3.1	0.8	0.2	12.5	3.1	0.8	0.2	0.5	0.0125
1[d]	35.0	35.0	–	31.2	–	–	–	>49T	>74T	>49T	27.9	–	–
12	33.0	–	27.8	–	22.0	15.3	11.3	2.1T	3.6	1.6	1.3	1.2	–
13	14.2	–	10.0					2.2	1.2	1.1	1.1	1.1	–
Mitomycin C	–	–	–	–	–	32.0	22.8	–	–	–	–	17.5T	8.6

[a] Conducted at Bristol Laboratories, Syracuse, N.Y. [b] For a detailed description of the procedure, see A. W. Bauer, W. M. Kirby, J. C. Sheris, and M. Turck, *Am. J. Clin. Pathol.*, **45**, 493 (1966). [c] For a detailed description of the procedure, see K. E. Price, R. E. Bush, and J. Lein, *Appl. Microbiol.*, **12**, 428 (1964). [d] Assays not concurrent but standardized against mitomycin C. T means toxic to culture; n means not active at the dose used; – means not tested at that dose.

a compound should show a ratio of test to control sample plaque counts of 3.0 or greater. Compound **12** barely meets this standard at a dose of 3.1 μg/mL (Table I), but compound **13** is not active at any dose used. In contrast, compound **1** and mitomycin C are highly active in the assay. Compounds **12** and **13** were not active against P388 murine leukemia according to the criterion of this assay, which requires a % T/C of 125 or greater.[10] In comparison, the 1-acetoxymitosene **1** was active at doses above 1.6 mg/kg (% T/C = 162 at 6.4 mg/kg optimal dose) and mitomycin C was active at doses above 0.2 mg/kg (% T/C = 283 at 6.4 mg/kg optimal dose).

The lack of significant activity for the quaternary ammonium compound **13** in the antitumor and λ-phage induction assays is disappointing in view of the presence of a good leaving group at position 1. However, it is possible that this compound does not reach the DNA receptor in high enough concentration. Since it is a cationic species, cell penetration might be difficult. We expect to prepare additional 1-substituted mitosenes in order to determine the most desirable properties for a substituent at this position.

Experimental Section

Melting points were determined on a Thomas-Hoover capillary apparatus. Infrared spectra were determined in KBr disks on a Beckman IR-33 spectrophotometer, nuclear magnetic resonance spectra were recorded on a Varian EM-360 spectrometer using tetramethylsilane as a standard, and ultraviolet spectra were recorded on a Cary-17 spectrophotometer. Elemental analyses were performed by the Microanalytical Laboratory, Department of Chemistry, Purdue University. Analytical results were within ±0.4% of the theoretical values. Each compound had IR and NMR spectra compatible with its structure.

2,3-Dihydro-7-methoxy-6-methyl-1-(*N*-pyrrolidino)-1*H*-pyrrolo[1,2-*a*]indole (6). Method A. Via Catalytic Reduction of the Corresponding Enamine. To a refluxing solution of 2.75 g (12.8 mmol) of the tricyclic ketone 5[7] and 4.3 mL (3.65 g, 51.2 mmol) of pyrrolidine in 275 mL of anhydrous benzene was added 2 g of Amberlyst 15 acidic ion-exchange resin (Mallinckrodt, sulfonic acid form). A Dean-Stark trap was used to collect water produced by the reaction. After 12 h the reaction was cooled and filtered. The filtrate was concentrated under reduced pressure and the residue was dissolved in a minimum of 1:1 methanol–ethyl acetate, treated with 200 mg of 5% palladium on charcoal, and shaken under 50 psi of hydrogen for 1 h. The catalyst was removed by filtration and the filtrate was concentrated to a dark oil. This oil was purified by chromatography on silica gel, using chloroform to elute impurities, and then 2–3% of isobutylamine was added to the solvent to elute the product. A light yellow oil that slowly solidified was obtained after evaporation of the solvent. Recrystallization from petroleum ether gave 1.93 g (56%) of the amine 6 as pale tan crystals: mp 102–103.5 °C; monomaleate salt mp 181–181.5 °C (from ethanol–ethyl acetate); IR (KBr) free base 2940, 2760, 1460, 1430 cm^{-1}; NMR free base ($CDCl_3$) δ 7.03 (s, 2, aromatic H's), 6.20 (s,1, aromatic H), 4.35–3.55 (m, 6, C-1 CH, C-3 CH_2, Ar-OCH_3 singlet at 3.84), 3.10–2.40 (broad signal, 6, C-2 CH_2, α-pyrrolidine CH_2's), 2.35 (s, 3, Ar-CH_3), 1.95–1.50 (broad signal, 4, β-pyrrolidine CH_2's).

Method B. Via Sodium Cyanoborohydride Reductive Amination. To a solution of 10.0 mL (8.52 g, 120 mmol) of pyrrolidine in 150 mL of methanol was added 8.0 mL (39.3 mmol) of 5 N hydrochloric acid in methanol, 4.15 g (19.3 mmol) of the tricyclic ketone **5**, and 1.373 g (21.8 mmol) of sodium cyanoborohydride. The resulting suspension was stirred in a closed vessel at room temperature for 44 h.

The mixture was made strongly acidic (pH 1–2) with concentrated hydrochloric acid, stirred open in the hood for 30 min, and evaporated under reduced pressure. The residue was diluted to 400 mL with water and washed with ethyl acetate (3 × 100 mL). The aqueous phase was made strongly basic (pH 11–12) with 10% sodium hydroxide and extracted with ethyl acetate (3 × 100 mL). The combined extracts were washed with 5% sodium carbonate (2 × 100 mL) and saturated sodium chloride solution (2 × 100 mL). The organic phase was dried and concentrated to a brown solid residue. The amine **6** was isolated from this residue as the monomaleate salt: $C_{17}H_{22}N_2O \cdot C_4H_4O_4$. This salt was identical (no depression of mixture melting point) with the monomaleate salt from method A: yield 5.70 g (76.3%); mp 184–185 °C (2-butanone). Recovery of 331 mg of ketone **5** was obtained from the mother liquor.

2,3-Dihydro-5,8-dioxo-7-methoxy-6-methyl-1-(*N*-pyrrolidino)-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (7). A solution of 729 mg (2.12 mmol) of **9** in 65 mL of tetrahydrofuran was treated with 459 mg of 10% palladium on charcoal and shaken with 70 psi of hydrogen for 20 h. After filtration the solution was concentrated and the residual oil was dissolved in 50 mL of acetone and added to 90 mL of 0.3 M, pH 6.7 phosphate buffer. This solution was treated with 2.36 g (8.80 mmol) of potassium nitrosodisulfonate and the mixture was stirred at room temperature for 1 h. It was diluted with water and extracted with methylene chloride (4 × 50 mL). The combined extracts were washed with 5% sodium carbonate (2 × 100 mL) and water (1 × 250 mL), dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a viscous brown oil. Column chromatography of this oil on 15 g of silica gel, using ethyl acetate as solvent, produced an intense orange band which, upon collection and evaporation, gave 502 mg (72%) of compound **7** as an oil that slowly crystallized.

The analytical sample, obtained by recrystallization from absolute ethanol, was glistening, dark orange red crystals with mp 139.5–140.5 °C; IR (neat on the oil) 2970, 2820 (CHO), 1680 (aldehyde C=O), 1660 and 1640 (quinone C=O's), 1580 cm^{-1}; NMR ($CDCl_3$) δ 10.45 (s, 1, CHO), 4.38 (m, 3, C-1 CH, C-3 CH_2), 4.13 (s, 3, Ar-OCH_3), 2.90–2.25 (broad signal, 6, C-2 CH_2, α-pyrrolidine CH_2's), 2.00 (s, 3, Ar-CH_3), 1.93–1.50 (broad signal, 4, β-pyrrolidine CH_2's).

2,3-Dihydro-7-methoxy-6-methyl-8-nitro-1-(*N*-pyrrolidino)-1*H*-pyrrolo[1,2-*a*]indole-9-carboxaldehyde (9). Dry *N*,*N*-dimethylformamide (10 mL) in an ice bath was treated with 3.9 mL of freshly distilled phosphorus oxychloride (6.53 g, 42.7 mmol). After 15 min the resulting solution was treated with 4.143 g (19.7 mmol) of the maleate salt of **6** in 70 mL of dry *N*,*N*-dimethylformamide, and the mixture was stirred for 20 h, initially at 0 °C and then at room temperature. It was poured into a solution of 10 g (121.95 mmol) of sodium acetate in 300 mL of water and stirred at room temperature of 3 h. The resulting solution was made basic (pH 11) with 10% sodium hydroxide and extracted with ethyl acetate (3 × 100 mL). The combined extracts were backwashed with water (4 × 200 mL), dried over anhydrous

sodium sulfate, and concentrated to give **8** as an oil that solidified (3.080 g, 96.6%): IR (neat) 2950, 2800 (CHO), 1640 cm^{-1} (C=O); NMR ($CDCl_3$) δ 10.01 (s, 1, CHO), 7.79 (s, 1, aromatic H), 6.98 (s, 1, aromatic H), 4.53 (t, 1, C-1 CH), 4.17–3.50 (m, 5, C-3 CH_2, Ar-OCH_3 at 3.90), 2.90–2.06 (broad signal plus singlet, 9, C-2 CH_2, α-pyrrolidine CH_2's, Ar-CH_3 at 2.30), 1.90–1.35 (broad signal, 4, β-pyrrolidine CH_2's).

A portion of this sample of **8** (779 mg, 2.61 mmol) was dissolved in 20 mL of glacial acetic acid and treated with 1 mL (excess) of 90% nitric acid. The resulting solution was stirred 16 h at room temperature, made strongly basic (pH 11–12) with 10% sodium hydroxide, and extracted with ethyl acetate (4 × 50 mL). The combined extracts were dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a brown residue. Removal of traces of solvent by extended vacuum pumping gave 825 mg (92%) of nearly pure **9**.

Recrystallization of a sample from absolute ethanol gave glistening yellow needles with mp 189–190 °C dec; IR (KBr) 2970, 2950, 2810 (CHO), 1670 (C=O), 1540 cm^{-1} (NO_2); NMR ($CDCl_3$) δ 10.03 (s, 1, CHO), 7.28 (s, 1, aromatic H), 4.83 (t, 1, C-1 CH), 4.23 (t, 2, C-3 CH_2), 3.90 (s, 3, Ar-OCH_3), 2.90–2.10 (br m plus s, 9, C-2 CH_2, α-pyrrolidine CH_2's, Ar-CH_3 at 2.43), 1.90–1.45 (broad signal, 4, β-pyrrolidine CH_2's).

2,3-Dihydro-5,8-dioxo-9-hydroxymethyl-7-methoxy-6-methyl-1-(*N*-pyrrolidino)-1*H*-pyrrolo[1,2-*a*]indole (**11**). A solution of 1.00 g (3.05 mmol) of **7** in 60 mL of 1:1 absolute ethanol–tetrahydrofuran under a nitrogen atmosphere was cooled to 0 °C in an ice bath. The cold solution was treated with 1.182 g (31.2 mmol) of sodium borohydride and stirred at 0 °C until the red color of the solution faded (approximately 45 min). The solution was treated with 10 mL (7.857 g, 135.5 mmol) of acetone and then 7 mL (7 mmol) of 1.0 M ferric chloride in 0.1 N hydrochloric acid. The mixture was diluted to 250 mL with water and extracted with methylene chloride (4 × 50 mL). The combined extracts were washed with saturated sodium chloride solution (1 × 150 mL), 10% sodium hydroxide (1 × 50 mL), and water (1 × 200 mL). The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure to give a deep red oil. This oil was chromatographed on a column of 25 g of silica gel (ethyl acetate as solvent) and the product was collected as a red-orange fraction. Evaporation of the solvent yielded an oil that crystallized on standing to give 654 mg (65%) of **11**.

The analytical sample was recrystallized from ethyl acetate to give fine, dull orange crystals: mp 127–128 °C; IR (neat) 3200 (OH), 2960, 2820, 1655 and 1645 cm^{-1} (quinone C=O's); NMR ($CDCl_3$) δ 4.85 (s, 2, C-10 CH_2), 4.58 (t, 1, C-1 CH), 4.30 (t, 2, C-3 CH_2), 4.02 (s, 3, Ar-OCH_3), 2.82–2.30 (br complex t, 6, C-2 CH_2, α-pyrrolidine CH_2's), 1.97 (s, 3, Ar-CH_3), 2.10–1.55 (broad signal, 4, β-pyrrolidine CH_2's).

2,3-Dihydro-5,8-dioxo-9-hydroxymethyl-7-methoxy-6-methyl-1-(*N*-pyrrolidino)-1*H*-pyrrolo[1,2-*a*]indole Carbamate [7-Methoxy-1-(*N*-pyrrolidino)mitosene, 12]. Phenyl chloroformate (2 mL) was added quickly to a stirred solution of 377 mg (1.14 mmol) of **11** in 40 mL of pyridine. After 2 h, the excess phenyl chloroformate was decomposed by the slow addition of water; the reaction was diluted to 150 mL with water and extracted with chloroform (3 × 25 mL). The combined extracts were washed with 5% sodium carbonate solution (2 × 50 mL) and water (3 × 100 mL). The organic phase was concentrated under reduced pressure, and the residual water and pyridine were removed under reduced pressure as an azeotrope by two treatments with toluene. The residue was chromatographed on a column of 15 g of neutral silica gel. Chloroform eluted a brown impurity and then the phenyl carbonate ester was eluted with ethyl acetate. Concentration of the orange eluate gave 236 mg of a syrup, which was converted directly to mitosene **12**.

A solution of 236 mg (0.525 mmol) of the syrup in 30 mL of methylene chloride was cooled in a dry ice–acetone bath, and ammonia was bubbled through the stirred solution until approximately 70 mL of ammonia had condensed (15–20 min). The solution was allowed to warm slowly, with ammonia evaporation, until the volume had reduced to that of the methylene chloride solution. This solution was washed with three equal volumes of water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure to a yellow-orange solid. Column chromatography of this solid on 10 g of neutral silica gel, using ethyl acetate initially as eluent and then 2:3 acetone–ethyl acetate, gave a red-orange fraction which, after evaporation of the solvent, yielded 125 mg (65%) of **12**.

An analytical sample was prepared by recrystallization from absolute ethanol as orange powdery crystals with mp 174–175 °C dec; IR (KBr) 3440 and 3370 (NH_2), 2950, 1720 (carbamate C=O), 1660 and 1645 cm^{-1} (quinone C-O's); NMR ($CDCl_3$) δ 5.28 (s, 2, C-10 CH_2), 5.03 (br s, 2, NH_2), 4.30 (br t, 3, C-1 CH, C-3 CH_2), 4.05 (s, 3, Ar-OCH_3), 2.58 (broad signal, 6, C-2 CH_2, α-pyrrolidine CH_2's), 1.97 (s, 3, Ar-CH_3), 1.78 (br s, 4, β-pyrrolidine CH's).

2,3-Dihydro-5,8-dioxo-9-hydroxymethyl-7-methoxy-6-methyl-1-(*N*-pyrrolidino)-1*H*-pyrrolo[1,2-*a*]indole Carbamate Methiodide [7-Methoxy-1-(*N*-pyrrolidino)mitosene Methiodide, 13]. To a solution of 120 mg (0.322 mmol) of the mitosene **12** in 25 mL of tetraydrofuran was added 1 mL (2.28 g, 16.1 mmol) of methyl iodide. The mixture, in a stoppered flask, was wrapped to exclude light and stirred at room temperature for 5.5 h. It was diluted with 15 mL of diethyl ether and filtered. The solid product was washed with ether and 2-butanone and allowed to air-dry to give 140 mg (85%) of orange powder.

The analytical sample, which decomposed without melting above 170 °C, was recrystallized from 95% ethanol–ethyl acetate: IR (KBr) 3430 and 3300 (NH_2), 2955, 1735 (carbamate C=O), 1665 and 1645 cm^{-1} (quinone C=O's); NMR (Me_2SO-d_6) δ 6.75 (s, 2, NH_2), 5.37 (s, 2, C-10 CH_2), 5.14 (t, 1, C-1 CH), 4.40 (broad signal, 2, C-3 CH_2), 4.00 (s, 3, Ar-OCH_3), 3.65 (broad signal, 4, α-pyrrolidine CH_2's), 3.14 (broad signal, 2, C-2 CH_2), 2.90 (s, 3, $^+NCH_3$), 2.17 (broad signal, 4, β-pyrrolidine CH_2's), 1.93 (s, 3, Ar-CH_3).

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