Synthesis and Antimicrobial Activity of Certain 6*H*-1,2,4-Oxadiazin-3(2*H*)-ones

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Treatment of 6*H*-1,2,4-oxadiazin-3(2*H*)-one-5(4*H*)-thione (**2**) with hydroxylamine, hydrazine, methylamine, or benzylamine afforded the corresponding N^5-substituted 5-amino-6*H*-1,2,4-oxadiazin-3(2*H*)-ones **3c–f**. Refluxing a dioxane solution of 6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**1**) with benzylamine or aminodiphenylmethane and hexamethyldisilazane in the presence of ammonium sulfate gave 5-benzylamino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (**3f**) and the corresponding 5-diphenylmethylamino derivative **3g**. Reaction of **1** with methyl iodide, benzyl chloride, dihydropyran, dihydrofuran, or benzyloxycarbonyl chloride afforded the corresponding 2-substituted 6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-diones **6a–e**. Reaction of 2-methyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**6a**) or the corresponding 2-benzyl derivative **6b** with phosphorus pentasulfide in dioxane gave 2-methyl-6*H*-1,2,4-oxadiazin-3(2*H*)-one-5(4*H*)-thione (**8a**) and the corresponding 2-benzyl derivative **8b**, respectively. Reaction of **8a** with ammonia in dioxane afforded 2-methyl-5-amino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (**9**). The degree of in vitro activity and the presence of antibacterial activity in the urine of animals given 5-amino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (**3a**) by oral route of administration prompted selection of this compound for further study.

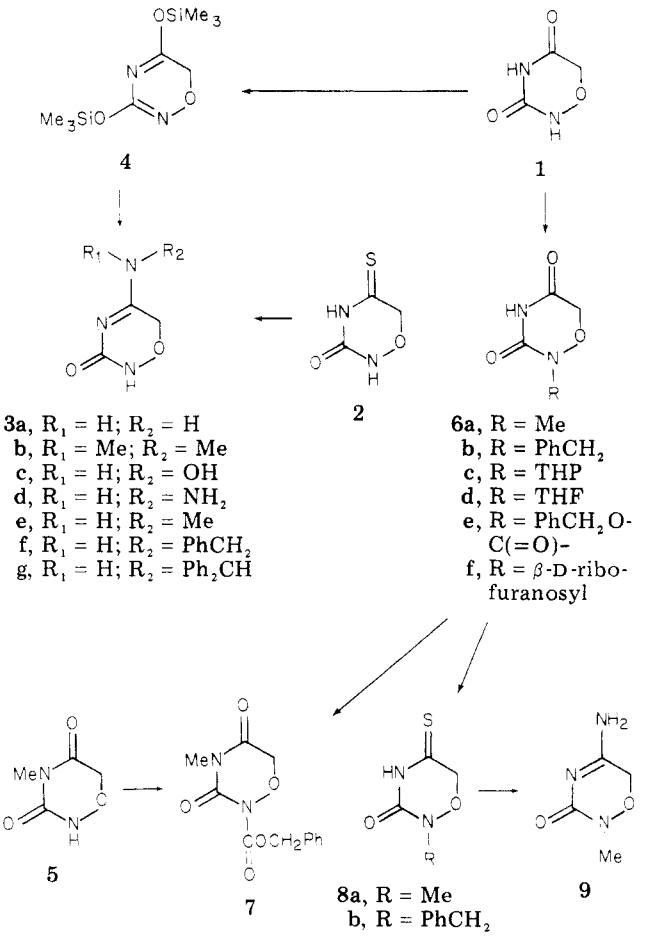
6*H*-1,2,4-Oxadiazine-3,5(2*H*,4*H*)-dione (**1**) has been shown to significantly inhibit growth in several bacterial systems while not being appreciably inhibitory to mammalian cells.[1] Although **1**, the 6-oxa analogue of uracil, is actually an isostere of 5,6-dihydrouracil, such that **1** can be considered as 6-oxadihydrouracil, the antibacterial activity of **1** is reversed by uracil and not by 5,6-dihydrouracil.[1] 6*H*-1,2,4-Oxadiazine-3,5(2*H*,4*H*)-dione (**1**) is, therefore, an apparent competitive antagonist of uracil in bacterial systems. This study was undertaken to further investigate the antimicrobial properties of the 6-oxa analogues of other pyrimidines. We have recently reported[2] the synthesis of the 6-oxa analogues of 4-thiouracil, cytosine, *N*,*N*-dimethylcytosine, and uridine. We discuss here the antibacterial, antifungal, and antiviral activities of these compounds as well as some newly prepared 6*H*-1,2,4-oxadiazin-3(2*H*)-ones.

Chemistry. Treatment of 6*H*-1,2,4-oxadiazin-3(2*H*)-one-5(4*H*)-thione (**2**)[2] in dioxane at room temperature with hydrazine, methylamine, or benzylamine, or reaction of **2** in ethanol at room temperature with hydroxylamine, afforded the corresponding N^5-substituted 5-amino-6*H*-1,2,4-oxadiazin-3(2*H*)-ones **3c–g** (Scheme I).

6*H*-1,2,4-Oxadiazine-3,5(2*H*,4*H*)-dione (**1**) could also be converted to 5-benzylamino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (**3f**) by conversion in situ to 3,5-bis(trimethylsilyloxy)-6*H*-1,2,4-oxadiazine (**4**) and subsequent reaction with benzylamine. Thus, refluxing a dioxane solution of **1**, hexamethyldisilazane, and benzylamine in the presence of ammonium sulfate afforded **3f** in 53% yield. When aminodiphenylmethane was used in place of benzylamine, 5-diphenylmethylamino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (**3g**) was obtained in 74% yield.

It had previously been reported[3] that reaction of 6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**1**) with methyl iodide in aqueous ethanol in the presence of sodium hydroxide afforded 4-methyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**5**). However, when we repeated the above alkylation, using the same conditions, we obtained 2-methyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**6a**). The structure of the alkylation product was assigned as **6a** by showing that this product was identical with an authentic sample of **6a** and different than an authentic sample of **5** by comparison of TLC, melting point, uv, ir, ^{1}H NMR, and pK_a (Table I). Cyclization of ethyl N^2-methylureidooxyacetate, MeNHC(=O)N(H)OCH$_2$CO$_2$Et, and ethyl N^1-methylureidooxyacetate, H$_2$NC(=O)N(Me)OCH$_2$CO$_2$Et, with sodium ethoxide in ethanol afforded **5** and **6a**, respectively.[4]

Scheme I



Reaction of **1** with benzyl chloride, using the same conditions as for the synthesis of **6a**, afforded 2-benzyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**6b**) (Scheme I). Reaction of **1** with dihydropyran or dihydrofuran in DMF in the presence of bis(*p*-nitrophenyl)phosphate afforded 2-(tetrahydro-2-pyranyl)-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**6c**) and 2-(tetrahydro-2-furanyl)-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**6d**), respectively. The structures of **6b–d** were assigned as the N-2 rather than the N-4 derivatives by comparison of their pK_a values with those of **5** and **6a** (Table I).

While reaction of **1** with acid chlorides, sulfenyl chlorides, phenyl isocyanate, or formaldehyde afforded the corresponding monosubstituted derivatives of **1**, whether

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Table I. Properties of 6H-1,2,4-Oxadiazin-3(2H)-ones

Compd	pK_a	Uv (EtOH) λ_{max}, nm ($\epsilon \times 10^{-3}$)	Ir, cm^{-1} [a]	^1H NMR,[b] C-6 ppm
1	7.6	220 (1 250)	3170, 3070, 1745, 1710	4.47
5	7.8	c	3250, 1735, 1680	4.61
6a	9.1	225 (1 640)	3170. 3070, 1725	4.61
6b	8.9	223 (3 400)	3180, 1745, 1715	4.60
6c	8.8	222 (1 890)	3200, 1730	4.58
6d	8.9	222 (2 030)	3200, 1730	4.60
6e		c	3190, 3090, 1765, 1725	4.77
7		c	1800, 1760, 1740, 1710	4.89
3a	6.1	228 (13 200)	3250, 1620	4.33
3b		245 (16 400)	3110, 1645	4.60
3c		217 (11 700)	3160, 1680	4.49
3d		210 (4 720)	3300, 3150, 1740	4.38
3e		233 (15 100)	3240, 3150, 1665	4.30
3f		237 (19 300)	3140, 1660	4.39
3g		239 (19 300)	3210, 1665	4.47
9	6.8	230 (12 500)	3270, 1645	4.48
2	7.1	274 (17 300)	3190, 1720	4.68
8a	8.0	275 (15 000)	3200, 1690	4.81
8b		277 (15 300)	3170, 1685	4.80

[a] KBr pellet. [b] Me_2SO-d_6. [c] End absorption only.

Table II. Carbon-13 Chemical Shifts of Some 6H-1,2,4-Oxadiazin-3(2H)-ones

Compd	Chemical shift, ppm[a] C-3	C-5	C-6	CH_3	Cbz C=O
6a	155.2	169.2	69.7	35.0	
8a	151.1	202.5	76.1	35.0	
6e	149.5	169.2	70.7		147.6
7	149.2	167.8	70.9	26.3	147.9

[a] Chemical shifts are measured from Me_2SO-d_6 and are converted to the Me_4Si scale using the relationship $\delta_{Me_4Si} = \delta_{Me_2SO\text{-}d_6} + 39.5$ ppm.

the substituent group was on N-2 or N-4 was not established.[5] Reaction of 1 with benzyloxycarbonyl chloride in the presence of sodium hydroxide afforded 2-benzyloxycarbonyl-6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (**6e**). Reaction of **6e** with methyl iodide in benzene in the presence of DBU afforded 2-benzyloxycarbonyl-4-methyl-6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (**7**). Reaction of 4-methyl-6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (**5**) with benzyloxycarbonyl chloride in the presence of sodium hydroxide also gave **7**, thus establishing **6e** as the N-2 rather than the N-4 substituted derivative.

In order to corroborate the structural assignments based on chemical evidence, carbon-13 nuclear magnetic resonance (^{13}C NMR) spectral data were obtained and are summarized in Table II. The assignment of the chemical shifts for **6a** have been previously reported.[2] The assignment of the carbonyl resonance of the benzyloxycarbonyl group of **6e** and of **7** was made by comparison with that of the benzyloxycarbonyl group of *N*-benzyloxycarbonylphthalimide which occurred at 147.7 ppm. The C-3 resonance of **6e** was shifted upfield by 5.7 ppm relative to **6a** while the C-5 resonance remained unchanged. This indicated that **6e** is the 2-benzyloxycarbonyl derivative. The similarity of the ^{13}C NMR spectra of **6e** and **7** provides additional confirmation for the assigned

Table III. In Vitro Antimicrobial Activity of 6H-1,2,4-Oxadiazin-3(2H)-ones and Ethyl Ureidooxyacetate (10)

Compd	MIC, μmol/ml[a,b] *E.c.*	*P.a.*	*S.a.*	*T.m.*	*C.a.*
1	0.01	0.04	0.02	>0.4	>0.4
6e	0.02	0.02	0.08	>0.4	>0.4
10	0.16	0.32	0.08	>0.4	>0.4
2	<0.005	0.02	0.01	0.32	0.32
8a	>0.4	>0.4	>0.4	0.16	0.4
8b	>0.4	>0.4	>0.4	0.16	0.32
3a	0.01	0.08	0.32	>0.4	>0.4
3b	0.16	>0.4	0.32	>0.4	>0.4
3c	>0.4	>0.4	>0.4	>0.4	>0.4
3d	>0.4	>0.4	>0.4	>0.4	>0.4
3e	0.32	>0.4	0.32	0.32	>0.4
3f	0.32	>0.4	0.4	>0.4	>0.4
3g	0.32	0.32	0.16	>0.4	>0.4

[a] Compound concentrations: 0.4-0.005 μmol/ml. [b] *Escherichia coli* (*E.c.*), *Pseudomonas aeruginosa* (*P.a.*), *Staphylococcus aureus* (*S.a.*), *Trichophyton mentagrophytes* (*T.m.*), *Candida albicans* (*C.a.*).

structure of **6e**.

Reaction of 2-methyl-6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (**6a**) or 2-benzyl-6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (**6b**) with phosphorus pentasulfide in refluxing, anhydrous dioxane afforded 2-methyl-6H-1,2,4-oxadiazin-3(2H)-one-5(4H)-thione (**8a**) and 2-benzyl-6H-1,2,4-oxadiazin-3(2H)-one-5(4H)-thione (**8b**), respectively. The structures of **8a** and **8b** were expected to be the 5-thio rather than the 3-thio derivatives since thiation of 6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (1) afforded 6H-1,2,4-oxadiazin-3(2H)-one-5(4H)-thione (**2**).[2] That thiation had in fact occurred at C-5 was confirmed by the use of ^{13}C NMR (Table II). The significant downfield shift of the C-5 resonance (33.3 ppm) of **8a** as compared to the C-5 resonance of **6a** is very similar to the shift observed for the C-5 resonance of **2** upon thiation.[2]

Reaction of 2-methyl-6H-1,2,4-oxadiazin-3(2H)-one-5-(4H)-thione (**8a**) in dioxane with ammonia at room temperature afforded 2-methyl-5-amino-6H-1,2,4-oxadiazin-3(2H)-one (**9**).

Biological Results. The antimicrobial activity of the 6H-1,2,4-oxadiazin-3(2H)-ones synthesized for this study is summarized in Table III. In addition to the antibacterial activity previously reported[1] for 6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (1) against *Streptococcus*, *Leuconostoc*, *Lactobacillus*, and *Escherichia*, this compound was found to inhibit *Pseudomonas* and *Staphylococcus*. Broad-spectrum antibacterial activity was maintained by substitution of the 5 position of 1 to produce either 6H-1,2,4-oxadiazin-3(2H)-one-5(4H)-thione (**2**) or 5-amino-6H-1,2,4-oxadiazin-3(2H)-one (**3a**). In contrast, 2-benzyloxycarbonyl-6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (**6e**) was the only N-2 substitution of 1 that did not result in a loss of antibacterial activity. All N-2 derivatives of **2** or **3a** were inactive as was the N-4 methyl derivative of 1. The N^5-substituted 5-amino-6H-1,2,4-oxadiazin-3(2H)-ones (**3b–g**) were significantly less inhibitory to bacteria than **3a**.

Antifungal activity against *Candida* and *Trichophyton* by **2** was not changed appreciably by N-2 substitution. The only N^5-substituted 5-amino-6H-1,2,4-oxadiazin-3(2H)-one that had antidermatophyte activity was 5-methylamino-6H-1,2,4-oxadiazin-3(2H)-one (**3e**). The antifungal activity of these compounds was not considered sufficient to warrant further study.

Ethyl ureidooxyacetate (**10**), from which 6H-1,2,4-oxadiazine-3,5(2H,4H)-dione (1) is synthesized,[1] has the same

spectrum of antibacterial activity as **1**, but it is four to sixteen times less active. It is interesting that the activity of **10** is reversed by uracil and uridine, as is the activity of **1**. This suggests that **10** and **1** may have the same site of action but that **1** is a more effective form. On the other hand, **10** could be as active as **1** but is transported into the cell less efficiently. Other alternatives such as ring opening or closure by the bacterium offer additional dimensions for mode of action studies.

All of the 6*H*-1,2,4-oxadiazin-3(2*H*)-ones in this study lacked antiviral activity against type 1 herpes simplex, type 3 parainfluenza, and type 13 rhino.

Urine was recovered from mice given a single, oral, 100 mg/kg dose of 6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**1**), 2-benzyloxycarbonyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**6e**), 6*H*-1,2,4-oxadiazin-3(2*H*)-one-5(4*H*)-thione (**2**), or 5-amino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (**3a**). Only urine recovered from animals given **3a** contained sufficient antibacterial activity to produce clear zones of inhibition.

Conclusions

It was of interest to find that strong in vitro antibacterial activity could be recovered in the urine of animals following oral administration of 5-amino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (**3a**). This compound was selected for further in vitro and in vivo chemotherapeutic evaluation because of its in vitro antibacterial activity and potential metabolic stability.

Experimental Section

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 60 MHz on a Hitachi Perkin-Elmer R-20A spectrometer in Me_2SO-d_6 using DSS as an internal standard. The ^{13}C NMR spectra were obtained on a Bruker HX-90 NMR spectrometer operating at 22.62 MHz in the Fourier transform mode at a probe temperature of 35 °C. A fabri-Tek 1074 signal averager with 4096 word memory was used for data accumulation and a PDP-8/e computer for data processing. Solutions (1.0 M) were prepared in Me_2SO-d_6 and were studied in 10-mm tubes. Ultraviolet spectra (uv, $\epsilon \times 10^{-3}$) were recorded on a Cary Model 15 spectrophotometer and infrared spectra (ir) on a Perkin-Elmer 257 spectrophotometer (KBr pellets). Elemental analyses were performed by Galbraith Laboratories, Inc., Knoxville, Tenn. The pK_a determinations were performed on a Radiometer automatic potentiometric titrator. Evaporations were carried out under reduced pressure below 40 °C. Detection of components on silica gel (ICN-Woelm F254) was by ultraviolet light.

Antimicrobial. The compounds synthesized for this study were assayed for antimicrobial activity using strains of *Escherichia coli* (*E.c.*), *Pseudomonas aeruginosa* (*P.s.*), *Staphylococcus aureus* (*S.a.*), *Candida albicans* (*C.a.*), and *Trichophyton mentagrophytes* (*T.m.*) isolated in the clinic. In vitro sensitivity of these organisms to this series of 6*H*-1,2,4-oxadiazin-3(2*H*)-ones was quantitatively determined by broth dilution assay. Serial dilutions were prepared in chemically defined medium in a range from 0.4 to 0.005 μmol/ml. The minimal inhibitory concentration (MIC) was recorded as the highest dilution of compound which prevented visible growth of the pathogen. Bacterial and yeast MIC's were read following 24 h of incubation at 35 °C. Dermatophyte inhibition was read after 48 h of incubation at 30 °C.[6]

Urine was recovered from mice (Cox, ICR strain) for 24 h after each animal received a single, oral, 100 mg/kg dose of test compound. Filter paper disks were saturated with urine and applied to the surface of agar growth medium seeded with a bacterium sensitive to the test compound. Disked plates were incubated for 18–24 h at 35 °C. A clear zone of inhibition surrounding the disk indicated the presence of antibacterial activity in the urine. In vitro antiviral activity was determined by the procedure of Sidwell and Huffman.[7]

5-Hydroxylamino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (3c). To 100 ml of an ethanolic solution of hydroxylamine [4.17 g (60 mmol) of NH_2OH–HCl was added to a solution of 1.38 g (60 mmol) of Na in 100 ml of EtOH; after stirring at room temperature for 2 h, the resulting suspension was filtered] was added 1.32 g (10 mmol) of **2**. After stirring at room temperature for 1 h, at which time uv indicated the absence of starting material, the solvent was removed in vacuo from the resulting yellow suspension to give a white solid residue which was then further dried in vacuo (vacuum pump). The residue was extracted with boiling EtOH (40 ml, 100 ml, and 50 ml) and the combined extracts were allowed to crystallize to give 0.669 g of a white crystalline solid. Further concentration of the mother liquor gave 0.125 g more product for a combined yield of 0.794 g (60.6%). Recrystallization from EtOH gave the analytical sample: mp 175–176 °C; NMR (Me_2SO-d_6) δ 4.49 (s, 2, CH_2), 9.80 (br s, 1, OH), 10.35 (br s, 2, NH). Anal. Calcd for $C_3H_5N_3O_3$ (131.091): C, 27.49; H, 3.85; N, 32.05. Found: C 27.39; H, 4.08; N, 32.28.

5-Hydrazino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (3d). To 0.448 g (3.4 mmol) of **2** in 7 ml of dry dioxane, cooled in an ice bath, was added dropwise 0.12 g (3.6 mmol) of anhydrous NH_2NH_2 in 2 ml of dry dioxane. The ice bath was removed and stirring was continued for 25 min. The resulting precipitate was filtered and washed well with ether to give 0.324 g (73.2%) of white solid. Several recrystallizations from EtOH gave the analytical sample: mp 170–171 °C; NMR (Me_2SO-d_6) δ 4.38 (s, 2, CH_2), 6.25 (br s, 2, NH_2), 11.40 (br s, 2, NH). Anal. Calcd for $C_3H_6N_4O_2$ (130.107): C, 27.70; H, 4.65; N, 43.06. Found: C, 27.77; H, 4.57; N, 42.85.

5-Methylamino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (3e). Methylamine was bubbled into a solution of 0.331 g (2.5 mmol) of **2** in 10 ml of dry dioxane for 5 min. After stirring at room temperature for 40 min, the resulting precipitate was filtered and washed with a little dioxane and then washed well with ether to give 0.315 g (97.6%) of white solid. Two recrystallizations from EtOH gave the analytical sample: mp 170.5–171.5 °C; NMR (Me_2SO-d_6) δ 2.83 (s, 3, NMe), 4.30 (s, 2, CH_2), 8.35 (br s, 1, N*H*Me), 9.80 (br s, 1, NH). Anal. Calcd for $C_4H_7N_3O_2$ (129.119): C, 37.21; H, 5.46; N, 32.54. Found: C, 37.17; H, 5.46; N, 32.49.

5-Benzylamino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (3f). A. To a solution of 0.396 g (3.0 mmol) of **2** in 10 ml of dry dioxane was added 0.343 g (3.2 mmol, 0.35 ml) of benzylamine. After stirring at room temperature for 1.5 h, the resulting precipitate was filtered and washed with a little dioxane and then washed well with ether to give 0.475 g (77.2%) of white solid. Recrystallization from 1:4 EtOH–Et_2O gave the analytical sample: mp 134–135 °C; NMR (Me_2SO-d_6) δ 4.39 (s, 2, CH_2), 4.56 (d, 2, NCH_2), 7.40 (s, 5, Ph), 8.80 (br s, 1, N*H*-Bzl), 9.84 (br s, 1, NH). Anal. Calcd for $C_{10}H_{11}N_3O_2$ (205.217): C, 58.53; H, 5.40; N, 20.48. Found: C, 58.41; H, 5.52; N, 20.40.

B. A solution of 0.580 g (5.0 mmol) of 6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (**1**), 5 mg of ammonium sulfate, 1.4 ml (13 mmol) of benzylamine, and 10 ml of HMDS in 20 ml of dry dioxane was refluxed for 15 h. After cooling, the dioxane and HMDS were removed in vacuo and the residue was then dried at the vacuum pump for 1 h. The residue was then suspended in petroleum ether and mixed well, and the petroleum ether was decanted. The residue was dissolved in 25 ml of dioxane and refrigerated. After a short time the resulting precipitate was filtered and washed well with ether to give 0.55 g (53.6%) of white solid, which is the same as the product obtained in A by comparison of TLC, melting point, ir, and NMR.

5-Diphenylmethylamino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (3g). A solution of 1.16 g (10 mmol) of **1**, 5 mg of ammonium sulfate, 5 ml (29 mmol) of diphenylmethylamine, and 20 ml of HMDS in 40 ml of dry dioxane was refluxed for 17 h. After cooling, dioxane and HMDS were removed in vacuo and the syrupy residue was dried at the vacuum pump for 1.5 h. The resulting semisolid residue was triturated with 100 ml of petroleum ether and filtered, and the white solid was washed well with petroleum ether to give 1.053 g. Recrystallization from EtOH gave the analytical sample: mp 203–204 °C dec; NMR (Me_2SO-d_6) δ 4.47 (s, 2, CH_2), 6.50 (d, 1, CH), 7.40 (s, 10, Ph), 9.20 (br d, 1, N*H*CHPh_2), 9.95 (br s, 1, NH). Anal. Calcd for $C_{16}H_{15}N_3O_2$ (281.315): C, 68.31; H, 5.37; N, 14.94. Found: C, 68.17; 5.42; N, 14.85.

The petroleum ether filtrate from above was concentrated in vacuo to a syrup, which was then triturated with $CHCl_3$ and filtered to give 0.852 g more product. This procedure was then repeated to give a further 0.172 g for a total yield of 2.077 g (73.9%).

2-Benzyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (6b). To a solution of 2.32 g (20 mmol) of **1** and 0.84 g (20 mmol) of NaOH in 40 ml of 1:1 EtOH–H_2O was added 2.6 g (20 mmol, 2.4 ml) of benzyl chloride. After heating on a steam bath for 3 h, the reaction mixture was cooled and the EtOH removed in vacuo. The aqueous solution remaining was extracted with ether (50 ml and 2 × 25 ml), and, after drying over Na_2SO_4, the ether was removed in vacuo to give 3.2 g of a syrup. Chromatography on 200 g of dry column silica gel, eluting with 400 ml of 97:3 $CHCl_3$–MeOH, gave 1.34 g (32.5%) of a syrup, which was crystallized from 1:6 $CHCl_3$–petroleum ether to give the analytical sample: mp 89 °C; NMR (Me_2SO-d_6) δ 4.60 (s, 2, CH_2), 4.76 (s, 2, CH_2Ph), 7.41 (s, 5, Ph), 11.30 (br s, 1, NH). Anal. Calcd for $C_{10}H_{10}N_2O_3$ (206.201): C, 58.25; H, 4.89; N, 13.59. Found: C, 58.48; H, 5.05; N, 14.63.

2-(Tetrahydropyran-2-yl)-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (6c). A solution of 5.80 g (50 mmol) of **1**, 8 ml (100 mmol) of dihydropyran, and 0.851 g (2.5 mmol) of bis(*p*-nitrophenyl) phosphate in 100 ml of DMF was heated on a steam bath for 20 h. After cooling, the DMF and excess DHP were removed in vacuo. The dark brown syrupy residue was taken up in 50 ml of EtOAc and washed first with 25 ml of saturated aqueous $NaHCO_3$ and then 25 ml of saturated aqueous NaCl solution. After drying over Na_2SO_4, the EtOAc was removed in vacuo and the residue taken up in 50 ml of $CHCl_3$. After 1 h the solid that had precipitated out was filtered off and the $CHCl_3$ removed in vacuo. The residue was then suspended in 100 ml of $CHCl_3$ and filtered, and the $CHCl_3$ was removed in vacuo to give 12.98 g of syrupy residue. Dry column chromatography on two 600-g columns (2.75 in. diameter), eluting each with 1000 ml of 9:1 $CHCl_3$–EtOAc, gave 5.7 g of slightly impure product as a syrup. Crystallization from $CHCl_3$–petroleum ether gave 4.9 g of a white solid. Recrystallization from $CHCl_3$–petroleum ether gave the analytical sample: mp 123–124 °C; NMR (Me_2SO-d_6) δ 1.3–2.1 (m, 6, $H_{2'}$, $H_{3'}$, and $H_{4'}$), 3.2–4.2 (m, 2, $H_{5'}$), 4.58 (s, 2, CH_2), 5.15 (m, 1, $H_{1'}$), 11.20 (br s, 1, NH). Anal. Calcd for $C_8H_{12}N_2O_4$ (200.194): C, 48.00; H, 6.04; N, 13.99. Found: C, 47.81; H, 5.86; N, 13.57.

The mother liquor was removed in vacuo and chromatographed on 90 g of dry column silica gel, eluting with 7:3 $CHCl_3$–EtOAc, to give 0.46 g of additional product for a combined yield of 5.4 g (53.9%).

2-(Tetrahydrofuran-2-yl)-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (6d). A solution of 0.580 g (5.0 mmol) of **1**, 0.70 g (10 mmol) of freshly distilled dihydrofuran, and 100 mg of bis(*p*-nitrophenyl) phosphate in 10 ml of DMF was heated in a bomb in a 60–75 °C bath for 15 h. The contents of the bomb were removed and the bomb was washed with $CHCl_3$. After removal of solvents in vacuo, the syrupy residue was chromatographed on 65 g of dry column silica gel, eluting with 100 ml of 7:3 $CHCl_3$–EtOAc, to give 0.364 g (39.1%) of a colorless syrup that soon solidified. Recrystallization from $CHCl_3$–petroleum ether gave the analytical sample: mp 114–115 °C; NMR (Me_2SO-d_6) δ 2.08 (m, 4, $H_{2'}$ and $H_{3'}$), 3.80 (m, 2, $H_{4'}$), 4.60 (s, 2, CH_2), 5.87 (t, J = 5 Hz, 1, $H_{1'}$), 11.2 (br s, 1, NH). Anal. Calcd for $C_7H_{10}N_2O_4$ (186.167): C, 45.16; H, 5.41; N, 15.05. Found: C, 45.43; H, 5.40; N, 14.68.

2-Benzyloxycarbonyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (6e). To a suspension of 1.16 g (10 mmol) of **1** and 0.45 g (11 mmol) of NaOH in 10 ml of H_2O, cooled in an ice bath, was added dropwise with vigorous stirring, 1.9 g (11 mmol) of benzyloxycarbonyl chloride. After stirring for 15 h, the white solid was filtered and the filtrate was extracted with petroleum ether (25 and 15 ml). The solid was taken up in 100 ml of CH_2Cl_2 and the aqueous filtrate was then extracted with this CH_2Cl_2 solution and then with an additional 25 ml of CH_2Cl_2. The combined CH_2Cl_2 extracts were dried over Na_2SO_4. Removal of the CH_2Cl_2 in vacuo gave 1.557 g (62.2%) of white solid. Two recrystallizations from CH_2Cl_2–petroleum ether gave the analytical sample: mp 128–129 °C; NMR (Me_2SO-d_6) δ 4.77 (s, 2, CH_2), 5.32 (s, 2, CH_2Ph), 7.47 (s, 5, Ph), 11.6 (br s, 1, NH). Anal. Calcd for $C_{11}H_{10}N_2O_5$ (250.210): C, 52.80; H, 4.03; N, 11.20. Found: C, 52.68; H, 3.99; N, 11.16.

2-Benzyloxycarbonyl-4-methyl-6*H*-1,2,4-oxadiazine-3,5(2*H*,4*H*)-dione (7). A. To a solution of 0.650 g (5.0 mmol) of **5** and 0.206 g (5.0 mmol) of NaOH in 5 ml of H_2O, cooled in an ice bath, was added dropwise with vigorous stirring 0.94 g (5.5 mmol) of benzyloxycarbonyl chloride. After stirring for 15 h, the reaction mixture was extracted with $CHCl_3$ (2 × 20 ml). After drying over $MgSO_4$, the $CHCl_3$ was removed in vacuo to give a colorless syrup, which was thoroughly triturated with petroleum ether to give, after drying in vacuo, 0.732 g (55.4%) of product which still contained a trace of benzyloxycarbonyl chloride. Preparative TLC of an aliquot on silica gel, eluting with 97:3 $CHCl_3$–MeOH, gave the analytical sample: NMR (Me_2SO-d_6) δ 3.11 (s, 3, 4-Me), 4.92 (s, 2, 6-CH_2), 5.37 (s, 2, PhCH_2), 7.51 (s, 5, Ph). Anal. Calcd for $C_{12}H_{12}N_2O_5$ (264.237): C, 54.55; H, 4.58; N, 10.60. Found: C, 54.23; H, 4.76; N, 10.27.

B. To a solution of 0.500 g (2.0 mmol) of **6e** and 0.34 g (2.2 mmol) of DBU in 25 ml of benzene was added 0.2 ml (3.2 mmol) of MeI. After refluxing for 2 h, the reaction mixture was allowed to cool to room temperature and was then extracted with 25 ml of H_2O and then 25 ml of saturated NaCl solution. After drying over $MgSO_4$, the benzene was removed in vacuo to give 0.371 g of a colorless syrup. Column chromatography on silica gel (20 g), eluting with 99:1 $CHCl_3$–EtOAc, afforded 0.138 g (26.1%) of a colorless syrup that was shown to be identical with the product obtained in A by comparison of NMR, ir, and TLC.

2-Methyl-6*H*-1,2,4-oxadiazin-3(2*H*)-one-5(4*H*)-thione (8a). A solution of 1.950 g (15 mmol) of **6a** and 3.5 g (15.8 mmol) of purified P_2S_5 in 75 ml of dry dioxane was refluxed for 2 h. After cooling, solvent was removed in vacuo and the resulting yellow syrup was extracted with 100 ml of ether. Filtration and removal of the solvent in vacuo gave 2.5 g of yellow solid, which was chromatographed on 400 g of dry column silica gel, eluting with 800 ml of 99:1 $CHCl_3$–MeOH, to give 0.727 g of homogeneous product. Recrystallization from 1:3 EtOH–petroleum ether gave the analytical sample: mp 116–117 °C; NMR (Me_2SO-d_6) δ 3.17 (s, 3, NMe), 4.81 (s, 2, CH_2), 12.82 (br s, 1, NH). Anal. Calcd for $C_4H_6N_2O_2S$ (146.169): C, 32.87; H, 4.14; N, 19.16; S, 21.94. Found: C, 33.04; H, 3.98; N, 18.99; S, 22.02.

A mixture fraction from the above chromatography was recolumned on 300 g of dry column silica gel, eluting with 450 ml of 98:2 $CHCl_3$–MeOH, to give 0.127 g more homogeneous product for a total yield of 0.854 g (39.0%).

2-Benzyl-6*H*-1,2,4-oxadiazin-3(2*H*)-one-5(4*H*)-thione (8b). A mixture of 0.606 g (2.9 mmol) of **6b** and 0.389 g (1.75 mmol) of purified P_2S_5 in 27 ml of dry dioxane was refluxed for 45 min. After cooling, the reaction mixture was filtered and the dioxane removed in vacuo. The residue was extracted with ether (2 × 25 ml) and the ether removed in vacuo to give 0.800 g of a yellow-brown syrup. Chromatography on a silica gel column (40 g), eluting with $CHCl_3$, gave 0.320 g (49.6%) of a homogeneous syrup. Crystallization from 1:9 $CHCl_3$–petroleum ether gave the analytical sample: mp 98 °C; NMR (Me_2SO-d_6) δ 4.80 (s, 4, CH_2 and CH_2Ph), 7.41 (s, 5, Ph), 12.93 (br s, 1, NH). Anal. Calcd for $C_{10}H_{10}N_2O_2S$ (222.266): C, 54.05; H, 4.54; N, 12.60; S, 14.42. Found: C, 54.14; H, 4.45; N, 12.56; S, 14.36.

2-Methyl-5-amino-6*H*-1,2,4-oxadiazin-3(2*H*)-one (9). Ammonia was bubbled into a solution of 0.291 g (2.0 mmol) of **8a** in 11 ml of dry dioxane for 1 h. The resulting precipitate was filtered and washed with a little dioxane and then washed well with ether to give 0.211 g (81.7%) of white solid. Recrystallization from MeOH and then EtOH gave the analytical sample: mp 166–167 °C; NMR (Me_2SO-d_6) δ 3.04 (s, 3, NMe), 4.48 (s, 2, CH_2), 7.90, 8.20 (br s, 2, NH_2). Anal. Calcd for $C_4H_7N_3O_2$ (129.120): C, 37.21; H, 5.46; N, 32.54. Found: C, 37.00; H, 5.47; N, 32.78.

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Mitomycin Antibiotics. Synthesis and Activity of 1,2-Disubstituted Mitosenes

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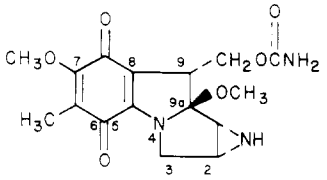
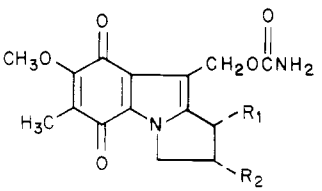
cis-1-Acetamido-2-acetoxy-7-methoxy-*N*-methylmitosene was prepared in 11 steps from 7-methoxy-6-methyl-2,3-dihydro-1*H*-pyrrolo[1,2-*a*]indol-1-one by a route involving bromination of the pyrrolidineenamine or trimethylsilyl enol ether of starting material, displacement of bromide by acetate, oxime formation, and reductive acetylation, followed by elaboration of the quinone and methyl carbamate functions according to previously established methods. An unsubstituted carbamate could not be prepared. The mitosene thus synthesized differs from previously reported 1,2-disubstituted mitosenes, which are derived from the solvolysis of mitomycins, in that it has the opposite arrangement of oxygen and nitrogen substituents at the 1 and 2 positions. It showed antibacterial activities in disk-plate assays superior to those of *cis*-diacetylapomitomycin A and equivalent to those of certain 1-substituted mitosenes; however, it was less active than mitomycin A in these assays. It was inactive in inducing λ-bacteriophage in *Escherichia coli* and inactive against P388 leukemia in mice. In contrast, certain 1-substituted mitosenes were active in prophage induction and **2b** and mitomycin A were active in both assays.

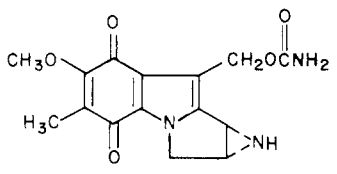
The mitosene family of compounds represents analogues (e.g., **2**) of the naturally occurring mitomycins in which the elements of methanol have been lost from the 9 and 9a positions.[1] Mitosenes are obtained from the mitomycins (for example, mitomycin A, 1) by procedures such as catalytic reduction followed by air oxidation[2] or acid-catalyzed solvolysis.[1,3] In addition, certain 1-substituted mitosenes have been prepared by total synthesis.[4] The biological activities of mitosenes depend upon substituents at the 7 position, which determine the ease of their reductive bioactivation,[5] and substituents at the 1 and 2 positions, which appear to involve binding to DNA.[6] Compounds such as 7-methoxymitosene (**2a**) and its 1-substituted analogues (e.g., **2c**) show antibacterial activities in culture, but they lack antitumor activity.[7] In contrast, 7-methoxyaziridinomitosene (**3**) has both antibacterial and antitumor activities, presumably because it is more reactive in binding to DNA after bioactivation.[5]



1



2a, $R_1 = R_2 = H$
b, $R_1 = OAc$; $R_2 = H$
c, $R_1 = OH$; $R_2 = H$
d, $R_1 = OH$; $R_2 = NH_2$
e, $R_1 = OH$; $R_2 = NHAc$
f, $R_1 = OAc$; $R_2 = NHAc$
g, $R_1 = OH$; $R_2 = NCH_3Ac$



3

Biological activities of 1,2-disubstituted 7-methoxymitosenes such as the *cis*-2-amino-1-hydroxy compound (apomitomycin A, **2d**) and its *N*-acetyl derivative **2e** appear to be somewhere between those of the aziridinomitosene (**3**) and the 1-substituted analogues; however, they have not been thoroughly studied. Thus, antibacterial activities in culture have been briefly reported for 1,2-disubstituted mitosenes by several groups.[5,7] The activity of **2e** against experimental sarcomas in mice was reported,[8,9] but no data on its activity against P388 and L1210 leukemia or other tumors were given. However, diacetyl derivative **2f** and *N*-methyl analogue **2g** showed very poor activity against L1210 leukemia.[10]

Since our continuing effort in the synthesis of mitomycin analogues had resulted in a reliable route to 1-substituted mitosenes,[4] we sought to extend this kind of route to 1,2-disubstituted mitosenes. The main problem in this extension appeared to lie in functionalizing the 2 position with a substituent that was stable to the chemical reactions required to elaborate the quinone and carbamate groups and that would lead to useful biological activity. The target compound of our synthesis became 1-acetamido-2-acetoxy-7-methoxymitosene (**15**, no methyl group on the carbamate nitrogen). This compound has an arrangement of 1- and 2-substituents opposite to that found in mitomycin solvolysis products such as **2d** and **2f**.[3] Hence, it is particularly interesting for structure–activity relationships. Earlier studies on pyrrolo[1,2-*a*]indoles had shown that in the 7-benzyloxy series of compounds it was possible to prepare the oxime of a 2-acetoxy 1-ketone.[11] If this same type of compound (**12**) could be prepared in the 7-methoxy-6-methyl series and reduced to the corresponding acetylated aminohydrin (**7**), we felt that there was a good chance that a synthesis of **15** could be achieved (Scheme I).

Starting with tricyclic ketone **4**,[4,12] the corresponding 2-bromo derivative **10** was prepared by way of the pyrrolidineenamine **5** in yields of 0–68%. As previously shown in the 7-benzyloxy series, direct bromination of ketones like **4** produces the 9-bromo derivative;[13] therefore, it is essential to activate the 2 position by making the enamine. In agreement with our previous report[4] the 7-methoxy-6-methylpyrrolo[1,2-*a*]indoles were less stable than their 7-benzyloxy analogues and special reaction conditions had to be developed. Thus, acid catalysts could not be used in preparing enamine **5**. Furthermore, hydrolysis of the

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