(NN=O); TLC (silica gel, 9:1 $CHCl_3$–MeOH, ninhydrin) showed two components, neither of which was the unnitrosated urea; ^1H NMR ($CDCl_3$–TMS) detected $ClCH_2CH_2NH$ attributable to the *N'*-nitroso isomer but showed much less OH than expected. A solution of this oil (5.00 g) in MeOH (100 ml) was stirred for 6 hr and left at 0° for 30 hr. Removal of the solvent under reduced pressure left a yellow oil, which was further dried in vacuo over P_2O_5 and eventually became a pasty solid when stored at ~0°: yield 4.52 g (85%, corrected for MeOH treatment); absence of nitrous ester shown by TLC and ir, which were identical with an analytical sample prepared similarly, but on a smaller scale; ^1H NMR showed the presence of some $ClCH_2CH_2NH$[13] but was otherwise consistent with the expected structure; ir (film) 3650–3050 (OH, NH), 1710 (C=O), 1520 (CNH), and 1485 cm^{-1} (N=O); mass spectrum (70 eV) *m/e* (rel intensity) 250 (0.02, $M^+ + 1$), 171 (0.1), 144 (1), 142 (20), 126 (0.1), 124 (17), 81 (100) (*m/e* 171, 144, and 126 could not reasonably derive from M, but could from iso-M, the *N'*-nitroso isomer—[iso-M − $Cl(CH_2)_2NH]^+$, [iso-M − $Cl(CH_2)_2NHCO + H]^+$, [iso-M − $Cl(CH_2)_2NHCO + H - H_2O]^+$). Anal. ($C_9H_{16}ClN_3O_3$) C, H, N.

Acknowledgment. This investigation was supported by the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare, through Contract No. NO1-CM-43762. The authors are indebted to Mrs. Martha Thorpe for interpretation of ^1H NMR spectra, to Mr. Marion C. Kirk for interpretation of mass spectra, and to Mrs. Ruby James for GC analyses.

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3-Substituted 2-Formylquinoxaline 1,4-Dioxides

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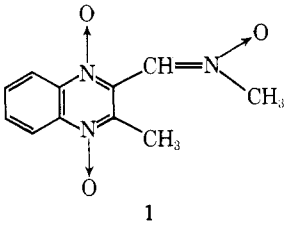
The methylnitrone of 3-methyl-1,4-dioxidoquinoxaline-2-carboxaldehyde (1) has shown exceptional antibacterial activity in vivo. Derivatives of 3-hydroxymethyl-1,4-dioxidoquinoxaline-2-carboxaldehyde and 3-acetoxymethyl-1,4-dioxidoquinoxaline-2-carboxaldehyde were prepared. Several of these compounds were found to be antibacterial agents of the same order of activity as 1.

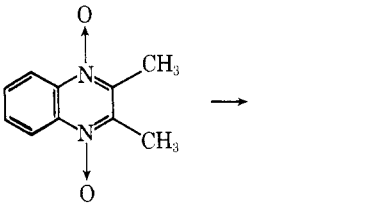
The methylnitrone of 3-methylquinoxaline-2-carboxaldehyde (1) has shown exceptional activity against *Proteus mirabilis* and *Salmonella schottmeulleri* in experimental



1

infections in mice.[1] The in vitro activity for 1 and its analogs is less than one would expect from their in vivo activity, suggesting the possible existence of an active metabolite.

Precedent for such an active metabolite in this type of series was found in a report on 2,3-dimethylquinoxaline 1,4-dioxide.[2] As with our compounds, the in vitro activity of 2 did not correlate well with the in vivo results. An investigation of the metabolism of 2 found 3 and 4 to be active metabolites.[3] Therefore, we felt the 3-hydroxymethyl analog of 1, if not a metabolite, was likely to be an active antibacterial and its synthesis was undertaken. The 3-acetoxymethyl analogs were also prepared as it was felt that in vivo these might be hydrolyzed to the hydroxymethyl compounds. A more likely explanation for the discrepancy between the in vivo and in vitro data for 1 appeared after this work was complete.[4] The in vitro antibacterial activity of quinoxaline 1,4-di-*N*-oxide (17) (Quidoxin, Imperial Chem-



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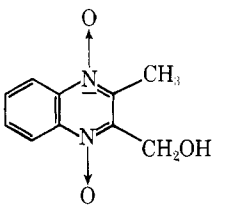

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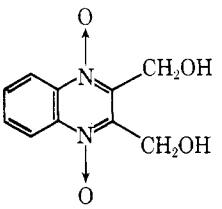

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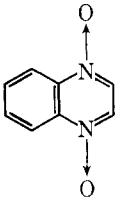

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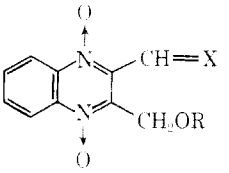
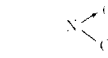
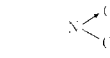
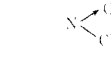
Table I. In Vivo Antibacterial Activity[a]



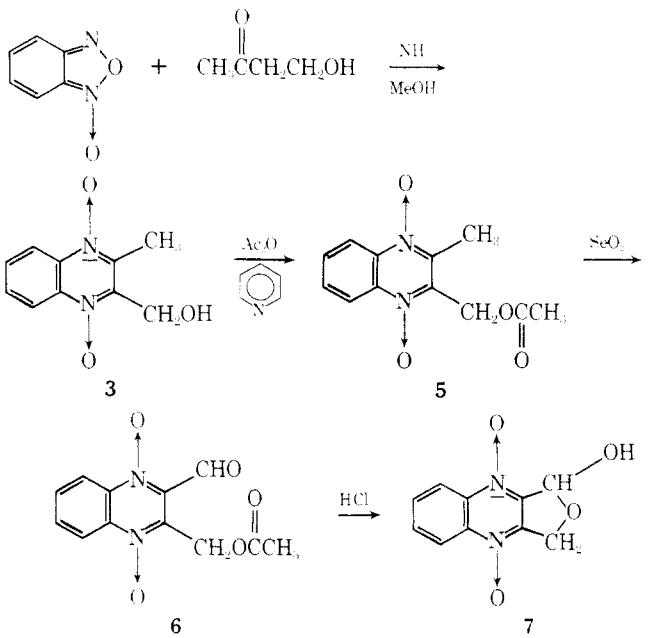
Compd no.	X	R	*S. aureus*[b] No. surv	*S. aureus*[b] Dose	*S. schott.*[c] No. surv	*S. schott.*[c] Dose	*P. mirabilis*[d] No. surv	*P. mirabilis*[d] Dose
6	O	$COCH_3$	2/5	50	4/5	50	5/5	250
7	O	H	0/5	250	5/5	50	8/10	250
8		$COCH_3$	3/5	50	5/5	50	10/10	25
9		H	NT		8/10	10	10/10	25
10		$COCH_3$	1/5	50	4/5	50	0/5	250
11	$NNHCONH_2$	H	2/5	250	0/5	250	2/5	50
12	$NNHCONH_2$	$COCH_3$	0/5	250	0/5	250	0/5	250
13	NOH	H	0/10	250	NT[e]		5/5	50
14	NOH	$COCH_3$	NT		NT		5/5	50
15		$COCH_3$	NT		NT		3/5	50
16	$NNHCSNH_2$	$COCH_3$	NT		NT		0/5	25
Streptomycin							10/10	10
Chloromycetin			5/5	50	5/5	50		

[a] Each compound was given to mice infected with a fatal infection. Dosages of 25, 50, or 250 mg/kg sc were given in four doses at times −4, +1, +20 and +24 hr. The challenge was given at 0 hr. The number of survivors is given with the dose level (mg/kg) for each organism. [b] *Staphylococcus aureus*. [c] *Salmonella schottmuelleri*. [d] *Proteus mirabilis*. [e] NT = not tested.

ical Industries, Ltd) is enhanced when determined under anaerobic conditions. This type of enhancement of activity has been observed with **1** when retested in vitro under conditions of reduced oxygen tension.[5] This suggests that an anaerobic or semianaerobic in vitro environment may more closely resemble the situation that exists in vivo.

The preparation of the acetoxymethyl- and hydroxymethylaldehydes (**7** and **8**) is outlined in Scheme I. The

Scheme I



reaction of **3** with acetyl chloride was attempted but resulted in a mixture of products, presumably due to reactions involving the *N*-oxide bonds. The use of acetic anhydride in pyridine gave **5** in 90% yield with no deoxygenation observed.

A series of derivatives of **6** and **7** was prepared which included nitrones (direct analogs of **1**) and other aldehyde derivatives such as semicarbazones and oximes. The acetoxymethylaldehyde **6** reacted with alkylhydroxylamines, hydroxylamine, and semicarbazide in the expected manner. The hydroxymethylaldehyde **7**, which exists as the hemiacetal, did not react with methylhydroxylamine to give isolable amounts of methylnitrone (**1**, R = OH) but reacted normally with hydroxylamine and semicarbazide. The preparation of the methylnitrone of **7** was achieved by hydrolysis of the ester **6**. The highly water-soluble product was isolated by use of Amberlite XAD-2 resin (see Experimental Section).

The two aldehydes and their derivatives (**6–16**, Table I) were screened in vitro and in vivo. The in vivo data are presented in Table I. As in the 3-methyl series, the methylnitrone is the most active derivative prepared. The less active compounds were also much less water soluble, which could account for some of the decreased activity. For the low activity of **10**, we have no explanation.

The activity of **8** and **9** is of the same order as that of **1**, but it does not seem to be such that one can claim **9** to be the active metabolite. A study of the metabolism of **1** did not indicate appreciable amounts of **9** to be formed.[6]

Experimental Section

Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. Infrared spectra were determined in pressed KBr disks. All compounds gave NMR, ir, and uv spectra

consistent with the proposed structure. All compounds gave analyses within ±0.4% of the theoretical values.

2-Hydroxymethyl-3-methylquinoxaline 1,4-Di-*N*-oxide (3). A mixture of benzofuroxan (54.4 g, 0.4 mol), 4-hydroxybutanone (40 g, 10% excess), and methanol (400 ml) was saturated with NH_3 gas. The mixture was stirred 1 hr after the exotherm subsided, chilled, and filtered. Recrystallization from methanol gave 30 g of tan solid, mp 182–184° (lit.[2] mp 182–184°).

2-Acetoxymethyl-3-methylquinoxaline 1,4-Di-*N*-oxide (5). A solution of **3** (30.9 g, 0.15 mol) in pyridine (500 ml) was chilled in an ice bath and Ac_2O (16 g, 0.15 mol) was added dropwise. The mixture was stirred overnight at room temperature, concentrated to 100 ml, and poured into ice water (500 ml). The aqueous mixture was filtered and the solid was dried to give 28 g of tan solid, mp 115–116°. The filtrate was extracted with $CHCl_3$ (4 × 200 ml). The combined organic extracts were extracted with aqueous HCl (2 × 100 ml), dried, and evaporated to an oil which solidified on trituration with isopropyl alcohol to give 5.4 g of solid, mp 115–116°. Recrystallization from benzene–hexane gave a yellow solid, mp 117–118°. Anal. ($C_{12}H_{12}N_2O_4$) C, H, N.

3-Acetoxymethylquinoxaline-2-carboxaldehyde 1,4-Di-*N*-oxide (6). A mixture of **5** (28 g, 0.113 mol), SeO_2 (12.5 g, 0.113 mol), and ethyl acetate (1.5 l.) was heated at reflux for 3 hr and filtered hot. The filtrate was chilled and then filtered to give 13.7 g of aldehyde. The filtrate, upon concentration to 500 ml and rechilling, gave another 10.8 g of aldehyde. Recrystallization from ethyl acetate gave a bright yellow solid, mp 157–159°. Anal. ($C_{12}H_{10}N_2O_5$) C, H, N.

1,3-Dihydrofuro[3,4-*b*]quinoxalin-1-ol 4,9-Di-*N*-oxide (7). A mixture of **6** (3.2 g, 0.12 mol) and dilute HCl (90 ml of H_2O/6 ml of concentrated HCl) was stirred at room temperature for 24 hr and filtered to give 2.8 g of product. Recrystallization from DMF gave the analytical sample, mp 215°. Anal. ($C_{10}H_8N_2O_4$) C, H, N.

α-(3-Acetoxymethylquinoxalin-2-yl)-*N*-methylnitrone 1,4-Dioxide (8). A suspension of **6** (10 g, 0.04 mol) and *N*-methylhydroxylamine oxalate (7.4 g, 0.04 mol) in ethanol (300 ml) was stirred while $NaHCO_3$ (6.7 g, 0.08 mol) was added in small portions. The mixture was heated at reflux for 2 hr and filtered hot and the filtrate was chilled to give 10.3 g of crude product. Recrystallization from ethanol gave 5.6 g of yellow solid, mp 169–170°. Anal. ($C_{13}H_{13}N_3O_5$) C, H, N.

α-(3-Hydroxymethylquinoxalin-2-yl)-*N*-methylnitrone 1,4-Di-*N*-oxide (9). A solution of **8** (2.9 g, 0.01 mol) in 100 ml of 1 *N* NaOH was stirred for 1 min and poured into 50 ml of water containing 0.6 g of acetic acid. The solution was concentrated to 50 ml at room temperature and then placed on a column of 300 g of Amberlite XAD-2. After 300 ml of water was collected off the column, the column was flushed with methanol. Three fractions were collected, fractions A and B consisting of 50 ml each and fraction C of 500 ml. Fraction B gave a bright yellow solid on chilling. Fraction C was evaporated and the bright yellow residue was recrystallized from isopropyl alcohol. The solids were combined to give 0.9 g of yellow solid, mp 141–143°. Anal. ($C_{11}H_{11}N_3O_4$) C, H, N.

α-(3-Acetoxymethylquinoxalin-2-yl)-*N*-(2-hydroxyethyl)-nitrone 1,4-Di-*N*-oxide (10). A mixture of **6** (10.8 g, 0.04 mol) and *N*-(2-hydroxyethyl)hydroxylamine oxalate (10.1 g, 0.04 mol) in ethanol (300 ml) was stirred while $NaHCO_3$ (6.7 g, 0.08 mol) was added in small portions. The mixture was stirred overnight and filtered. Two recrystallizations from nitromethane gave 2.8 g of bright yellow solid, mp 159–160°. Anal. ($C_{14}H_{15}N_3O_6$) C, H, N.

3-Hydroxymethylquinoxaline-2-carboxaldehyde 1,4-Di-*N*-oxide Semicarbazone (11). A mixture of **7** (4.4 g, 0.02 mol), semicarbazide hydrochloride (2.2 g, 0.02 mol), $NaHCO_3$ (1.7 g, 0.02 mol), and methanol (200 ml) was heated at reflux for 3 hr and filtered hot. The solid was washed with water and air-dried to give 3 g of yellow solid, mp 232°. Anal. ($C_{11}H_{11}N_5O_4$) C, H, N.

3-Acetoxymethylquinoxaline-2-carboxaldehyde 1,4-Di-*N*-oxide Semicarbazone (12). A mixture of **6** (5.2 g, 0.02 mol), $NaHCO_3$ (1.7 g, 0.02 mol), semicarbazide hydrochloride (2.2 g, 0.02 mol), and methanol (200 ml) was heated at reflux for 1 hr, chilled, and filtered. The solid was recrystallized from DMF to give 3.4 g of yellow solid, mp 250°. Anal. ($C_{13}H_{13}N_5O_5$) C, H, N.

3-Hydroxymethylquinoxaline-2-carboxaldehyde 1,4-Di-*N*-oxide Oxime (13). A suspension of **7** (7.4 g, 0.023 mol) and hydroxyamine hydrochloride (4.1 g, 0.06 mol) in methanol (600 ml) was heated at reflux until solution was achieved (30 min). The solution was chilled and filtered to give a solid which was recrystallized from methanol to give 5 g of yellow solid, mp 185–186°. Anal. ($C_{10}H_9N_3O_4$) C, H, N.

3-Acetoxymethylquinoxaline-2-carboxaldehyde 1,4-Di-*N*-oxide Oxime (14). A mixture of **6** (10.5 g, 0.04 mol), hydroxylamine hydrochloride (2.8 g, 0.04 mol), and $NaHCO_3$ (3.4 g, 0.04 mol) in methanol (500 ml) was heated at reflux for 30 min, chilled, and filtered. The solid was recrystallized from nitromethane to give 6 g of yellow solid, mp 177–178°. Anal. ($C_{12}H_{11}N_3O_5$) C, H, N.

3-[(3-Acetoxymethyl-1,4-dioxo-2-quinoxalinyl)methyl-$\Delta^{1,N}$-amino]-2-oxazolidinone (15). A mixture of **6** (10.4 g, 0.04 mol) and 3-amino-2-oxazolidone (4.1 g, 0.04 mol) in ethanol (300 ml) was heated at reflux for 30 min, chilled, and filtered. The solid was recrystallized two times from nitromethane to give 3.7 g of yellow solid, mp 238–239°. Anal. ($C_{15}H_{14}N_4O_6$) C, H, N.

3-Acetoxymethylquinoxaline-2-carboxaldehyde 1,4-Di-*N*-oxide Thiosemicarbazone (16). **6** (5.2 g, 0.02 mol) and thiosemicarbazide (1.8 g, 0.02 mol) were suspended in ethanol (300 ml) and the mixture was heated at reflux for 3 hr, chilled, and filtered. The solid was recrystallized from DMF to give 3.1 g of yellow solid, mp 236–237°. Anal. ($C_{13}H_{13}N_5O_4S$) C, H, N.

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