(3) J. S. G. Cox, *Nature (London)*, **216**, 1328 (1967).
(4) J. Goose and A. M. J. N. Blair, *Immunology*, **16**, 749 (1969).
(5) G. J. Possanza, A. Bauen, and P. B. Stewart, *Int. Arch. Allergy Appl. Immunol.*, **49**, 789 (1975).
(6) P. B. Stewart, unpublished results.
(7) W. E. Brocklehurst, *J. Physiol. (London)*, **151**, 416 (1960).
(8) L. C. Miller and M. L. Tainter, *Proc. Soc. Exp. Biol. Med.*, **57**, 261 (1944).

Synthesis and Antibacterial Activity of Isomeric 6- and 7-Acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-Dioxides

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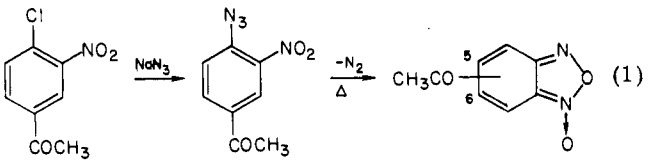
The synthesis, separation, and structure determination of 6- and 7-acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-dioxides are reported together with a comparison of their antibacterial activity. The structural assignment of these 6- and 7-acetyl isomers was based on NMR analysis of related mono-*N*-oxide derivatives, which were obtained by treatment of the quinoxaline 1,4-dioxides with acetic anhydride–acetic acid or trimethyl phosphite. The compounds were screened for in vitro and in vivo activity against *Escherichia coli*, *Salmonella choleraesuis*, *Pasteurella multocida*, and *Streptococcus pyogenes*. Although the isomers were found to possess similar activity, the 7-acetyl isomer was more active therapeutically in mice than the 6-acetyl isomer when administered parenterally.

A large number of quinoxaline 1,4-dioxides (QNO's) exhibit antibacterial activity.[1,2] Although the synthesis of some mixtures of 6- and 7-substituted QNO's has been reported,[3,4] only in a few cases has each isomer been isolated and characterized.[4] Furthermore, to our knowledge there are no studies reported where the antibacterial activity has been determined for both isomers of such a pair.[5] This paper describes the synthesis, separation, and structure determination of 6- and 7-acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-dioxides (**1** and **2**, respectively) and a comparison of their antibacterial activity.[6]

Synthesis. The starting material for the synthesis of compounds **1** and **2** was 5(6)-acetylbenzofurazan 1-oxide (**3**), prepared in an analogous manner to that reported for 5(6)-formylbenzofurazan 1-oxide by Ghosh and Whitehouse[7] (eq 1). The intermediate azide was synthesized by allowing 4-chloro-3-nitroacetophenone to react with sodium azide in dimethyl sulfoxide (Me_2SO) at room temperature. Thermal decomposition of the azide in refluxing toluene gave the desired benzofurazan 1-oxide (BFO) **3** in 74% overall yield.



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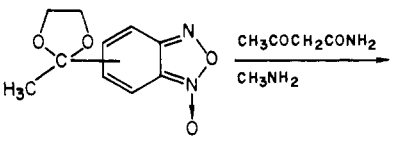
The 6- and 7-substituted QNO mixture **4**, containing a protected acetyl group, was isolated in 84% yield from the "Beirut reaction"[8] of 5(6)-acetylbenzofurazan 1-oxide ethylene ketal (**5**) and acetoacetamide (eq 2). The use of unprotected BFO **3** in this reaction led to low yields of **1** and tar formation apparently due to side reactions of the acetyl group.[9] Dilute acid hydrolysis of **4** gave a 1:1 mixture of the 6- and 7-acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-dioxides (**1** and **2**, respectively) that was readily separated by fractional crystallization.

In general, the structural assignment of 6- and 7-substituted QNO isomers is difficult since both isomers have very similar spectral properties. However, their conversion to mono-*N*-oxide derivatives allows structure determination by NMR;[10] in these, aromatic protons H-5 and H-8, unlike their counterparts in the parent compounds, are in a different chemical environment. For example, the 6-isomer **1** was converted to the 1- and 4-mono-*N*-oxides **6** and **7**, as shown in eq 3. The NMR spectrum of **1** possesses an H-5 doublet ($J_{H\text{-}5\text{–}H\text{-}7}$ = 2 Hz) at 0.38 ppm lower field than the multiplet for H-7 and H-8. After the loss of a neighboring *N*-oxide, i.e., in the formation of the 1-oxide **6** upon treatment of **1** with acetic anhydride–acetic acid,[11] H-5 moves upfield 0.25 ppm. The chemical shifts of protons H-7 and H-8 are unaffected. Reduction of **1** with trimethyl phosphite in refluxing 1-propranol[12] gave the 4-oxide **7**. The H-5 doublet is found at the same chemical shift as H-5 in the parent dioxide **1**.



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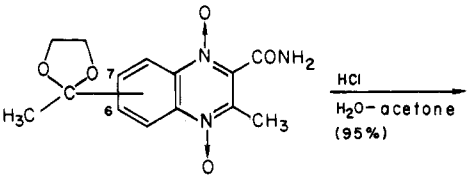

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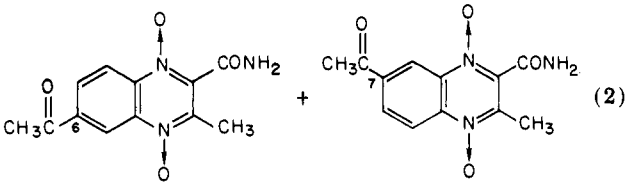

1 2

In contrast with what was observed with the 6-isomer **1**, the low-field doublet in the NMR spectrum of the 7-isomer **2** (H-8, $J_{H\text{-}6\text{–}H\text{-}8}$ = 2 Hz) remains at the same chemical shift after acetic anhydride–acetic acid rearrangement to the 1-oxide **8** as illustrated in eq 4. A portion of the H-5–H-6 multiplet moves upfield slightly. Reduction of **2** with trimethyl phosphite gave the 4-oxide **9**. The H-8 doublet moves upfield by 0.38 ppm relative

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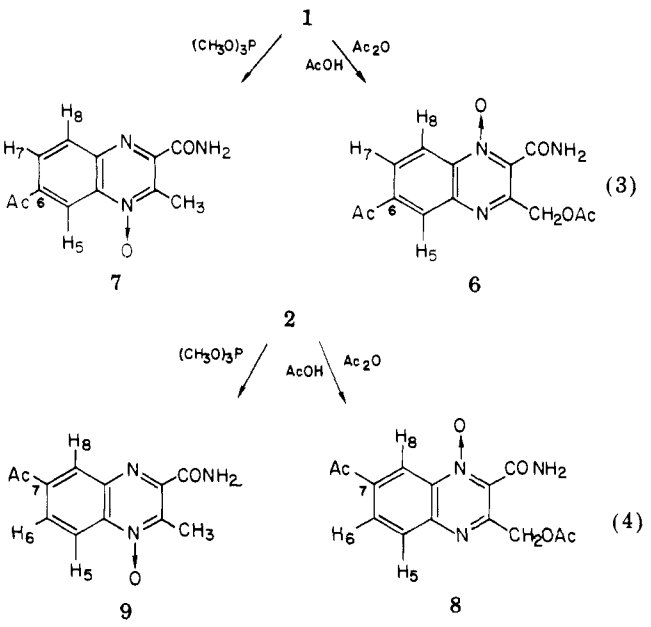

to H-8 in the parent dioxide **2**.

Biological Activity. Compounds **1** and **2** were evaluated for in vitro and in vivo antibacterial activity against gram-negative and gram-positive microorganisms. Both were found to possess excellent activity in these screens. As shown in Table I, within experimental error there is little difference between the activity of **1** and **2** when evaluated in vitro. However, compound **2** is significantly ($p < 0.001$) more active than compound **1** in vivo following parenteral administration. As illustrated in Table II, compound **2** has a lower PD_{50} value, when administered subcutaneously, against all the experimental infections. Since both the oral and parenteral activities of compound **1** are nearly equal within experimental error, it would appear that absorption of this compound is approximately equivalent regardless of the route of administration. With compound **2** we observed a ca. twofold decrease in efficacy when **2** was given orally, suggesting more rapid absorption of this compound following subcutaneous dosing.

Experimental Section

Experimental Infections in Mice. Male and female mice weighing 11–13 g obtained from Blue Spruce Farms, Alamont, N.Y., were used in all experiments. Acute systemic infections were produced by intraperitoneal inoculation of one to ten times the number of organisms necessary to kill 100% of the nonmedicated mice in 4 days. Standardized bacterial cultures of *Escherichia coli* and *Salmonella choleraesuis* were suspended in 5% hog gastric mucin, and *Pasteurella multocida* and *Streptococcus pyogenes* were suspended in brain–heart infusion broth. Treatment was initiated 0.5 h after infection. A second treatment was administered at 4.0 h and a third at 24 h. A 50% protective dose value (PD_{50}) was calculated by the probit method.[13]

Antimicrobial Susceptibility Tests. Minimum inhibitory concentrations (MIC) were determined by inoculating with a replicating device approximately 10^4 organisms onto brain–heart infusion agar containing the quinoxaline 1,4-dioxide in twofold dilutions.[14] Plates were incubated overnight at 37 °C in the anaerobic environment achieved in a Gas Pac. The MIC was taken to be the lowest concentration at which no growth appeared.

General. Melting points (uncorrected) were taken with a Thomas-Hoover capillary apparatus. NMR spectra were recorded on Varian A-60 and T-60 spectrometers with Me_4Si as internal standard. IR spectra were determined with a Perkin-Elmer Model 21 spectrophotometer. UV spectra were recorded on a Cary Model 14 spectrophotometer. Mass spectra were obtained with a Perkin-Elmer RMU-6E mass spectrometer. Microanalyses were performed by the Pfizer Analytical Department. All evaporations were conducted in vacuo using either a water aspirator or a vacuum pump.

Table I. In Vitro Antibacterial Activity

Microorganism	MIC,[a] μg/mL 1	2
E. coli	0.39	0.39
S. choleraesuis	0.39	0.19
P. multocida	0.39	0.19
Strep. pyogenes	0.19	0.39

[a] Minimum inhibitory concentration; determined under anaerobic conditions as described in the Experimental Section.

Table II. In Vivo Antibacterial Activity

Microorganism	PD_{50},[a] mg/kg 1	2
E. coli		
Oral	12.6 ± 4.4	13.0 ± 4.8
Subcutaneous	10.9 ± 1.8	5.6 ± 0.8
S. choleraesuis		
Oral	9.9 ± 1.5	9.1 ± 2.1
Subcutaneous	7.6 ± 1.1	4.5 ± 0.9
P. multocida		
Oral	13.6 ± 1.7	12.7 ± 2.2
Subcutaneous	11.7 ± 1.4	7.7 ± 1.9
Strep. pyogenes		
Oral	70 ± 27	15.0 ± 4.7
Subcutaneous	54 ± 13	9.2 ± 2.3

[a] Determined as described in the Experimental Section; 95% confidence limits.

5(6)-Acetylbenzofurazan 1-Oxide (3). A mixture of 4-chloro-3-nitroacetophenone (112 g, 0.56 mol) and sodium azide (36.5 g, 0.56 mol) in 675 mL of Me_2SO was stirred overnight at room temperature. The reaction mixture was poured into 2 L of water and the solution was extracted with seven 300-mL portions of toluene. The toluene extract was dried over anhydrous magnesium sulfate and then heated under reflux until nitrogen evolution had ceased (ca. 1 h). The solvent was removed under vacuum leaving a yellow solid, 74 g (74%) of **3**: mp 89–91 °C (mp 90–91 °C after recrystallization from MeOH); NMR ($CDCl_3$) δ 2.60 (3, s, CH_3CO), 7.4–8.1 (3, m, aromatic H's); IR (KBr) 1694 cm^{-1} (C=O); UV λ_{max} (MeOH) 243 nm (ε 21 500), 370 (6000); mass spectrum *m/e* 178 (M^+). Anal. ($C_8H_6N_2O_3$) C, H, N.

5(6)-Acetylbenzofurazan 1-Oxide Ethylene Ketal (5). To a solution of 5(6)-acetylbenzofurazan 1-oxide (**3**, 11.0 g, 0.062 mol) in 900 mL of toluene was added ethylene glycol (160 mL) and *p*-toluenesulfonic acid (0.7 g). The two-phase mixture was refluxed for 6 h and water was removed during the course of the reaction by means of a Dean-Stark trap. The reaction mixture was then cooled to room temperature, and the toluene layer was separated and washed four times with 300-mL portions of water and dried over anhydrous magnesium sulfate. The toluene was removed in vacuo, and the resulting solid was recrystallized from ether–hexane to give 10.3 g (83%) of **5** as yellow crystals: mp 80–81 °C; NMR ($CDCl_3$) δ 1.63 (3, s, CH_3), 3.8–4.2 (4, m, CH_2CH_2), 7.4–7.6 (3, m, aromatic H's); IR (KBr) no C=O absorption; mass spectrum *m/e* 222 (M^+). Anal. ($C_{10}H_{10}N_2O_4$) C, H, N.

6- and 7-Acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-Dioxide Ethylene Ketals (4). 5(6)-Acetylbenzofurazan 1-oxide ethylene ketal (**5**, 6.66 g, 0.03 mol) and acetoacetamide (3.03 g, 0.03 mol) were dissolved in 25 mL of tetrahydrofuran. Methylamine in water (1 mL, 40% solution) was added and the reaction mixture was stirred at room temperature 3 days. The pale yellow solid was collected by suction filtration and washed thoroughly with tetrahydrofuran to afford 7.68 g (84%) of **4**: mp 207–209 °C (mp 214–215 °C after recrystallization from MeOH); NMR ($CDCl_3$–CF_3CO_2D) δ 1.77 (3, s, CH_3), 2.87 (3, s, CH_3), 3.8–4.3 (4, m, CH_2CH_2), 7.6–8.9 (5, m, aromatic H's, NH_2); IR (KBr) 1695, 1725 cm^{-1}, (C=O's); UV λ_{max} (MeOH) 236 nm (ε 21 700), 269 (26 000), 382 (11 000); mass spectrum *m/e* 305 (M^+). Anal. ($C_{14}H_{15}N_3O_5$) C, H, N.

6-Acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-Dioxide (1) and 7-Acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-Dioxide (2). A mixture of the 6- and 7-acetyl-3-methyl-2-quinoxalinecarboxamide 1,4-dioxide ethylene ketals (**4**, 27 g, 0.089 mol) was dissolved in 2.5 L of acetone and 150 mL of 1 N hydrochloric acid, and the solution was refluxed for 5 h. During this time yellow crystals precipitated that were collected by suction filtration upon termination of the reaction. The crystals were washed thoroughly with acetone to afford 11.5 g (50%) of **2**: mp 229–230 °C; NMR (CH_3CO_2D) δ 2.97, 3.03 (6, two overlapping singlets, CH_3, $COCH_3$), 8.60–9.10 (2, m, H-5, H-6), 9.40 (1, d, J = 2 Hz, H-8); IR (KBr) 1680 ($CONH_2$), 1700 cm^{-1} ($COCH_3$); UV λ_{max} (MeOH) 238 nm (ϵ 24 200), 280 (23 300), 385 (8390); mass spectrum m/e 261 (M^+). Based on spectral data less than 10% of **1** was present in this sample of **2**. Anal. ($C_{12}H_{11}N_3O_4$) H, N; C: calcd, 55.22; found, 54.80.

The mother liquor was concentrated to 0.5 vol in vacuo and 10.4 g (45%) of **1** crystallized from the solution: mp 216–217 °C; NMR (CF_3CO_2D) δ 3.00 (6, s, CH_3, $COCH_3$), 8.60–9.10 (2, m, H-7, H-8), 9.47 (1, d, J = 2 Hz, H-5); IR (KBr) 1695 cm^{-1} (shoulder at 1690, $COCH_3$, $CONH_2$); UV λ_{max} (MeOH) 237 nm (ϵ 21 500), 278 (22 100), 380 (6330); mass spectrum m/e 261 (M^+). Based on spectral data, less than 10% of **2** was present in this sample of **1**.

5(6)-Acetylbenzofurazan 1-oxide (**3**, 1.78 g, 0.01 mol) and acetoacetamide (1.01 g, 0.01 mol) were dissolved in 25 mL of tetrahydrofuran. A solution of methylamine in methanol (1.0 mL of a 4.56 M solution) was added to the reaction mixture, which was then stirred at room temperature for 18 h. The dark reaction mixture was filtered under suction, and 0.50 g (19%) of **1** was obtained as yellow crystals which melted at 216–217 °C after Darco treatment and recrystallization from methanol. The reaction mother liquor contained tars. None of **2** was found to be present (<10%) in this sample of **1** based on spectral data. The mixture melting point with material (**1**) from above was 216–217 °C. Anal. ($C_{12}H_{11}N_3O_4$) C, H, N.

Quinoxaline Mono-*N*-oxides 6–9. These compounds were prepared solely for the structural elucidation of **1** and **2** and were used without purification in order to prevent fractionalization of 6- and 7-acetyl isomers. Selective monodeoxygenation of **1** and **2** to afford **7** and **9**, respectively, was accomplished in refluxing 1-propanol containing trimethyl phosphite in the manner described previously.[12] Acetic anhydride–acetic acid rearrangement of **1** and **2** to the corresponding 3-acetoxymethyl-2-quinoxalinecarboxamide 1-oxides **6** and **8**, respectively, was carried out according to a literature procedure.[11] Compounds **6–9** were characterized by NMR, IR, UV, and mass spectral analyses.

Acknowledgment. We are indebted to Dr. James W. McFarland for stimulating our interest in this problem, and we wish to thank Dr. Robert F. Myers for advice regarding this work. We thank Richard Pezzullo and Jules Aimetti for their technical assistance.

References and Notes

(1) (a) J. Francis, J. K. Landquist, A. A. Levi, J. S. Silk, and J. M. Thorp, *Biochem. J.*, **63**, 455 (1956); (b) J. R. Valenta, J. R. E. Hoover, and J. F. Pagano, *Antimicrob. Agents Chemother.*, 453 (1966); (c) E. N. Padeiskaya, G. N. Pershin, and T. P. Radkevich, *Int. Congr. Chemother., Proc., 5th*, 1, 825 (1967); *Chem. Abstr.*, **70**, 18594b (1969).

(2) Carbadox, a QNO, is highly effective as a growth promotant for swine: G. W. Thrasher, J. E. Shively, C. E. Askelson, W. E. Babcock, and R. R. Chalquest, *J. Anim. Sci.*, **31**, 333 (1970).

(3) (a) M. J. Haddadin, G. Agopian, and C. H. Issidorides, *J. Org. Chem.*, **36**, 514 (1971); (b) E. Abushanab and N. D. Alteri, Jr., *ibid.*, **40**, 157 (1975).

(4) (a) W. Dürckheimer, *Justus Liebigs Ann. Chem.*, **756**, 145 (1972); (b) N. A. Mufarrij, M. H. Haddadin, C. H. Issidorides, J. W. McFarland, and J. D. Johnston, *J. Chem. Soc., Perkin Trans. 1*, 965 (1972).

(5) It has generally been assumed, although not determined, that the antibacterial activity of both isomers would be similar: T. H. Cronin and K. Richardson, U.S. Patent 3 818 007 (1974).

(6) Subsequent to completion of our work a patent describing some of this work has been issued from these laboratories: U.S. Patent 3 948 911 (1976).

(7) P. B. Ghosh and M. W. Whitehouse, *J. Med. Chem.*, **11**, 305 (1968).

(8) (a) M. J. Haddadin and C. H. Issidorides, *Tetrahedron Lett.*, 3253 (1965); (b) *Heterocycles*, **4**, 767 (1976).

(9) A mixture of 6- and 7-substituted QNO isomers is expected when 5(6)-substituted BFO's are used (ref 3 and 4). However, the major isomer in this reaction is predicted to be the 6-acetyl QNO isomer **1**, as observed, based on previous studies with BFO's containing electron-withdrawing substituents (ref 3b).

(10) This technique has been used previously for the structural determination of other 6- and 7-substituted QNO isomers (ref 4a).

(11) M. J. Haddadin and A. S. Salameh, *J. Org. Chem.*, **33**, 2127 (1968).

(12) Our recent study of the reduction of some 2,3-disubstituted QNO's with trimethyl phosphite in refluxing alcohol solvent has shown that deoxygenation occurs exclusively at the nitrogen adjacent to the carbon bearing an electron-withdrawing group: J. P. Dirlam and J. W. McFarland, *J. Org. Chem.*, **42**, 1360 (1977).

(13) H. E. Batson, "An Introduction to Statistics in the Medical Sciences", Burgess Publishing Co., Minneapolis, Minn., 1957, pp 64–69.

(14) E. Steers, E. L. Foltz, and B. S. Graves, *Antibiot. Chemother.*, **9**, 307 (1959).

Quinolone Antimicrobial Agents. 1. Versatile New Synthesis of 1-Alkyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic Acids

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A flexible reaction sequence has been developed which starts with readily available anthranilic acids or isatoic anhydrides and leads regiospecifically to 1-alkyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acids after reaction with 1,3-dicarbonyl compounds. The sequence is superior to earlier published methods by allowing electron-releasing and -withdrawing groups in any position on the aromatic ring, by allowing convenient substitution at C_2, and better overall yield. A number of new and known antimicrobial agents were prepared and tested in vitro, demonstrating, inter alia, that substitution of the H at C_2 abolishes antibacterial activity.

Synthetic antimicrobial agents descended from nalidixic acid (**1**),[1] including oxolinic acid (**2**),[2] piromidic acid (**3**),[3] and pipemidic acid (**4**)[4] have found clinical acceptance in the treatment of human urinary tract infections. Closely

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