# Articles

## Pyridoquinoxaline *N*-Oxides. 1. A New Class of Antitrichomonal Agents[1]

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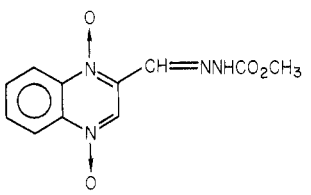
An aspect of our work with quinoxaline 1,4-dioxides involved the synthesis and reactivity of (quinoxalin-2-yl)acrylonitrile 1,4-dioxide. We have found that treatment of a methanolic solution of this unsaturated nitrile (cis or trans) with primary alkylamines affords a novel series of pyrido[2,3-*b*]quinoxaline 5-oxides. These tricyclic pyridoquinoxalines represent a unique class of agents with oral activity against trichomoniasis.

Continuing interest in the chemistry of quinoxaline 1,4-dioxides (QNO's)[2] is due in part to their importance as animal health products. For example, the QNO carbadox[3] (1) is effective as a growth promotant for swine and



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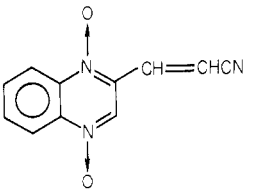

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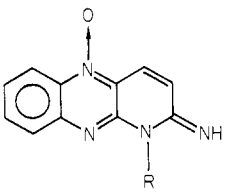
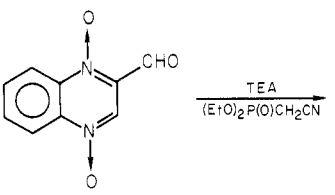


3

in the prevention of swine dysentery. An aspect of our work with QNO's involved the synthesis and reactivity of the structurally related quinoxalinylacrylonitrile **2**. We have found that treatment of **2** with primary amines affords a novel series of pyrido[2,3-*b*]quinoxaline 5-oxides (**3**). These tricyclic pyridoquinoxalines represent a unique class of agents with oral activity against trichomoniasis. The chemistry and antitrichomonal activity of these new heterocycles are the subjects of this paper.

**Chemistry.** The unsaturated nitrile **2** was viewed as a potential precursor to other quinoxaline *N*-oxides. Specifically, we were interested in examining the reactivity of Michael acceptors such as **2** toward amines. Synthesis of **2** involved treatment of the QNO aldehyde **4** with diethyl cyanomethylphosphonate in the presence of triethylamine (Scheme I). The nitrile was formed in good yield (70%) as a mixture (1:1) of cis (**2a**) and trans (**2b**) isomers, which were readily separated by fractional crystallization. Exposure of a methanolic solution of **2a** or **2b** to primary alkylamines did not give either of the expected Michael adducts (**5a,b**) (Scheme II). Rather, the products isolated under these conditions were shown to be the novel pyrido[2,3-*b*]quinoxaline 5-oxides **3** (see Table I).

Scheme I



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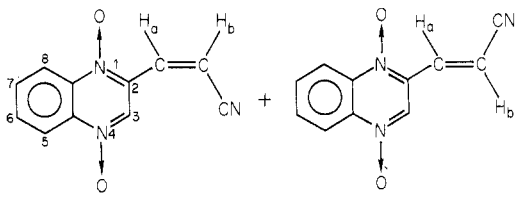
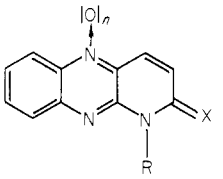


2a 2b

The reduction and base hydrolysis of the pyridoquinoxalines were readily accomplished (see Table II). Treatment of **3** with $TiCl_3$ in aqueous THF[2c] gave the corresponding deoxides **6**. Hydrolysis of **3** to the pyridones **7** was carried out by warming with ethanolic sodium hydroxide. Either **6** or **7** (R = $CH_3$) could be converted to the pyridone **8**. The mechanism of formation of **3** has not been examined in detail. However, production of **3** from either the cis or trans nitrile (**2a** or **2b**) (Scheme III) suggests that the reaction involves double-bond isomerization via reversible Michael addition of the amine (e.g., **2** ⇄ **5**). Further reaction is likely to involve attack of amine at the 3-position of **2** or **5**, followed by loss of water



**3**, R = $CH_3$, $CH_2CH_3$, 4-picolyl; $n$ = 1; X = NH
**6**, R = $CH_3$, $CH_2CH_3$, 4-picolyl; $n$ = 0; X = NH
**7**, R = $CH_3$, $CH_2Ph$; $n$ = 1; X = O
**8**, R = $CH_3$; $n$ = 0; X = O

(1) This paper has been presented in part. See "Abstracts of Papers", Second Chemical Congress of the North American Continent, Las Vegas, NV, Aug 1980, American Chemical Society, Washington, DC, Abstr MEDI 28.
(2) (a) J. P. Dirlam and J. W. McFarland, *J. Org. Chem.*, **42**, 1360 (1977), and references cited therein. (b) John P. Dirlam, Leonard J. Czuba, Beryl W. Dominy, Richard B. James, Richard M. Pezzullo, Joseph E. Presslitz, and Wendell W. Windisch, *J. Med. Chem.*, **22**, 1118 (1979). (c) B. W. Cue, Jr., J. . Dirlam, E. A. Glazer, *Org. Prep. Proc. Int.*, **9**, 263 (1977).
(3) Mecadox (Pfizer, Inc., New York, NY).

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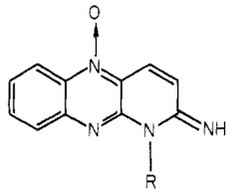
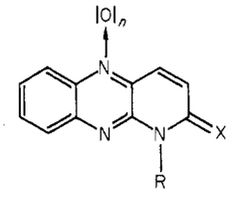
Table I. *N*-Alkylpyrido[2,3-*b*]quinoxalin-2(1*H*)-imine 5-Oxides (**3**)



| no. | R | mp, °C | yield,[a] % | formula | anal. |
|---|---|---|---|---|---|
| **3a** | $CH_3$ | 224-225 | 63 | $C_{12}H_{10}N_4O \cdot 0.75H_2O$ | C, H, N |
| **3b** | $CH_2CH_3$ | 188-189 | 50 | $C_{13}H_{12}N_4O$ | C, H, N |
| **3c** | $CH_2CH_2CH_3$ | 165-167 | 66 | $C_{14}H_{14}N_4O$ | C, H, N |
| **3d** | $CH_2Ph$ | 195-197 | 71 | $C_{18}H_{14}N_4O$ | C, H, N |
| **3e** | $CH_2CH{=}CH_2$ | 182-184 | 57 | $C_{14}H_{12}N_4O \cdot 0.20H_2O$ | C, H, N |
| **3f** | $CH_2CH_2OCH_3$ | 163-164 | 40 | $C_{14}H_{14}N_4O_2 \cdot 0.20H_2O$ | C, H, N |
| **3g** | $CH_2CH_2OCH_2CH_3$ | 171-172 | 50 | $C_{15}H_{16}N_4O_2$ | C, H, N |
| **3h** | cyclohexyl | 182-184 | 35 | $C_{17}H_{18}N_4O$ | C, H, N |
| **3i** | 4-picolyl | 195-197 | 60 | $C_{17}H_{13}N_5O$ | C, H, N |
| **3j** | $CH_2CH_2OH$ | 199-200 | 44 | $C_{13}H_{12}N_4O_2 \cdot 0.25H_2O$ | C, H, N |
| **3k** | $CH_2CH(OCH_3)_2$ | 172-173 | 60 | $C_{15}H_{16}O_3N_4$ | C, H, N |

[a] Yields after purification based on **2a** or **2b**.

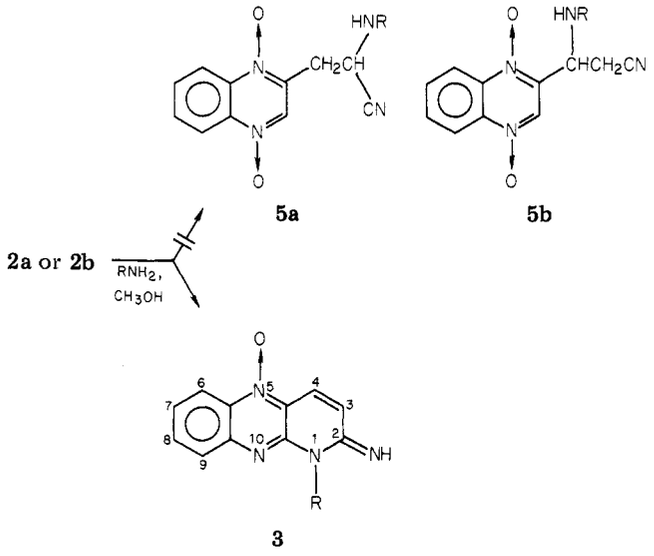
Table II. Reduction and Base Hydrolysis of **3**



| no. | X | R | *n* | mp, °C | yield,[a] % | formula | anal. |
|---|---|---|---|---|---|---|---|
| **6a** | NH | $CH_3$ | 0 | 157-159 | 32 | $C_{12}H_{10}N_4 \cdot H_2O$ | C, H, N |
| **6b** | NH | $CH_2CH_3$ | 0 | 128-130 | 60 | $C_{13}H_{12}N_4$ | C, H, N |
| **6c** | NH | 4-picolyl | 0 | 179-181 | 45 | $C_{17}H_{13}N_5$ | C, H, N |
| **7a** | O | $CH_3$ | 1 | 247-248 | 69 | $C_{12}H_9N_3O_2$ | C, H, N |
| **7b** | O | $CH_2Ph$ | 1 | 212-214 | 58 | $C_{18}H_{13}N_3O_2$ | C, H, N |
| **8** | O | $CH_3$ | 0 | 243-245 | 45 | $C_{12}H_9N_3O$ | C, H, N |

[a] Yields after purification.

Scheme II




to give the unsaturated 3-aminoquinoxaline 1-oxide **9a**. The latter can undergo intramolecular cyclization to the pyridoquinoxaline **3**. In support of this mechanism, we have demonstrated that **2** (**a** or **b**) reacts with secondary amines to give the 3-(dialkylamino)quinoxaline 1-oxides **9b,c**.

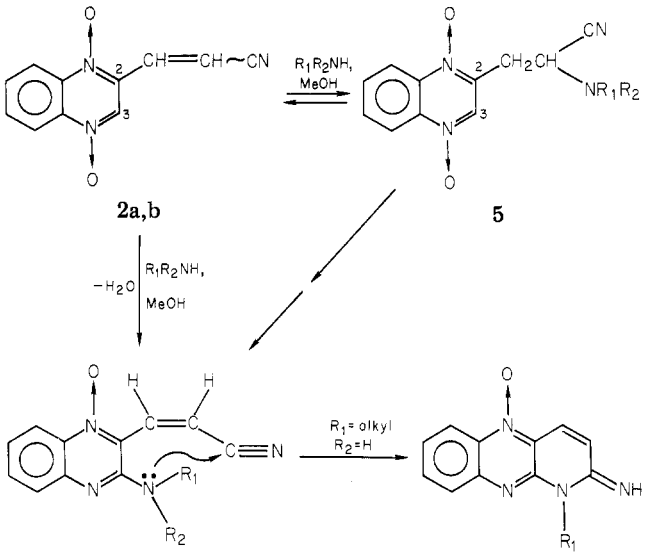
**Biological Activity.** Trichomoniasis is a protozoan infection of the human and bovine genitourinary tracts.[4] *Trichomonas vaginalis*, an etiologic agent of vulvovaginitis in women, and *Tritrichomonas foetus*, responsible for abortion in cattle, are the common pathogenic species. The most effective drugs currently available for treatment are the nitroimidazoles (e.g., tinidazole, metronidazole). The pyridoquinoxalines were evaluated orally against *T. foetus* in mice. Activity was based upon reduction in the severity of peritoneal cavity infection (see Table III). Tinidazole, the positive control, was consistently effective[5] down to 12.5 mg/kg (sid ×3). Several of the *N*-oxides (**3b,f,h,i**) were comparable to tinidazole at 25 mg/kg but were inactive at 12.5 mg/kg. However, the *N*-methyl analogue **3a** was completely effective at 12.5 mg/kg and is considered to be equipotent to tinidazole[6] against *T. foetus*. The deoxygenated pyridoquinoxalines **6a, b** retained substantial activity but were less effective than the corresponding *N*-oxides. Hydrolysis of the exocyclic carbon–nitrogen double bond in either **3a** or **6a** caused a sharp reduction in antitrichomonal activity (cf. pyridones **7a** and **8**).

## Experimental Section

**Biology. Infections in Mice.** Therapeutic antitrichomonal studies[7] were conducted in 6-week-old CF1 male mice weighing

(4) William J. Ross, in "Burger's Medicinal Chemistry", Part II, 4th ed., Manfred E. Wolff, Ed., Wiley-Interscience, New York, 1979, pp 460–461.
(5) Tinidazole reduced severity of peritonitis by 80–100% at 12.5, 25, or 50 mg/kg (sid ×3).
(6) The MEC of tinidazole against *T. foetus* in mice is reported[7] to be 12.5 mg/kg (sid ×3).

Scheme III



**9a**, $R_1$ = alkyl; $R_2$ = H
**b**, $R_1 = R_2 = CH_3$
**c**, $R_1, R_2 = (CH_2)_4$

Table III. Antitrichomonal Activity (*T. foetus*)

| no. | dose,[a] mg/kg | % reduction in peritonitis: compound | % reduction in peritonitis: tinidazole |
|---|---|---|---|
| **3a** | 50 | 98 | 97 |
| | 25 | 97 | 92 |
| | 12.5 | 100 | 95 |
| **3b** | 25 | 100 | 100 |
| | 12.5 | 0 | 100 |
| **3c** | 50 | 0 | NA[b] |
| **3d** | 25 | 19 | 78 |
| **3e** | 25 | 38 | 100 |
| **3f** | 25 | 96 | 100 |
| | 12.5 | 0 | 100 |
| **3g** | 50 | 9 | 89 |
| **3h** | 25 | 96 | 100 |
| | 12.5 | 21 | 100 |
| **3i** | 50 | 95 | NA[b] |
| | 25 | 81 | 89 |
| | 12.5 | 18 | 79 |
| **3j** | 25 | 57 | 96 |
| **3k** | 25 | 25 | 100 |
| **6a** | 50 | 98 | 89 |
| | 25 | 75 | 95 |
| | 12.5 | 34 | 85 |
| **6b** | 25 | 89 | 96 |
| | 12.5 | 0 | NA[b] |
| **6c** | 50 | 15 | 89 |
| **7a** | 25 | 23 | 100 |
| **8** | 25 | 18 | 93 |

[a] In mice, sid ×3. [b] Data not available.

approximately 20 g (Carworth Farms, Charles River Breeding Farms, Wilmington, MA). They were caged in groups of 8–10 and received intraperitoneally 3.5 to 7 × $10^6$ *Tritrichomonas foetus* (ATCC 30231) cultured in standard tryptocase serum base (STS) medium (BBL) plus 6% human serum. Drugs dissolved in water were administered orally in 0.5 mL volume at 50 or 25 mg/kg once daily for 3 days (sid ×3) beginning 1 day after infection. Mice were necropsied 1 day after the last treatment. Peritonitis induced by *T. foetus* was scored from 0 to 5 as follows: 0 = no peritoneal exudate or viable organisms; 1 = peritoneal exudate but no viable organisms; 2–4 = mild, moderate, or severe peritonitis with viable organisms; 5 = death due to trichomoniasis. Moderate to severe infections were induced in nonmedicated controls with an average score of 3.4 over 12 trials. Activity (Table III), expressed as percent reduction in severity of infection, was computed by dividing the score of each treated group by the corresponding score of the infected control. Drugs reducing peritonitis by at least 90% at 25 or 50 mg/kg were retested at 12.5, 25, and 50 mg/kg, and tabulated scores were averages of the two tests.

**Chemistry. General.** Melting points were determined with a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded on a Varian T-60 spectrometer 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. All compounds gave spectral data consistent with the proposed structure. Microanalyses were performed by the Pfizer Analytical Department.

**(Quinoxalin-2-yl)acrylonitrile 1,4-Dioxide (2a,b).** To a stirred suspension of 20.0 g (0.105 mol) 2-quinoxalinecarboxaldehyde 1,4-dioxide[8] and 18.6 g (0.105 mol) of diethyl cyanomethylphosphonate in 425 mL of methanol was added dropwise a solution of 10.6 g (0.105 mol) triethylamine in 30 mL of methanol. All starting material went into solution shortly after the addition of triethylamine was complete. Within 10 min a pale yellow solid precipitated and was collected by filtration. Trituration with methanol gave 8.1 g (36%) of **2b**, mp 222–223 °C. The NMR spectrum ($CF_3CO_2H$) of **2b** had characteristic absorptions at δ 7.6 (d, $J$ = 18 Hz, 1 H, CH=C*H*CN), 8.0 (d, $J$ = 18 Hz, 1 H, *CH*=CHCN), 8.35 (m, 2 H, H-6 and H-7), 8.95 (m, 2 H, H-5 and H-8), 9.5 (s, 1 H, H-3). Anal. ($C_{11}H_7N_3O_2$) C, H, N.

The mother liquor obtained from the filtration of **2b** was concentrated and cooled. The resulting precipitate was collected by filtration and triturated with cold methanol to give 7.6 g (34%) of **2a**, mp 170–172 °C. The NMR spectrum ($CDCl_3$) of **2a** had absorptions at δ 6.0 (d, 1 H, $J$ = 12 Hz, CH=C*H*CN), 7.9 (m, 3 H, C*H*=CHCN, H-6 and H-7), 8.62 (m, 2 H, H-5 and H-8), 9.05 (s, 1 H, H-3). Anal. ($C_{11}H_7N_3O_2$) C, H, N. The infrared spectra (KBr) of both **2a** and **2b** had nitrile absorptions at 2222 $cm^{-1}$.

**1-Methylpyrido[2,3-*b*]quinoxalin-2(1*H*)-imine 5-Oxide (3a).** A slurry of 1.0 g (4.7 mmol) of either **2a** or **2b** in 600 mL of methanol was perfused with methylamine for 2 h.[9] The resulting light brown solution was stoppered and allowed to stand for 16 h at 20 °C. The solvent was evaporated under reduced pressure, leaving an orange solid. Recrystallization from methanol gave 709 mg (63%) of **3a**, mp 224–225 °C. The NMR spectrum ($CDCl_3$) had absorptions at δ 3.70 (s, 3 H, N-C*H*$_3$), 6.82 (d, 1 H, $J$ = 8 Hz, H-3), 7.20 (d, 1 H, $J$ = 8 Hz, H-4), 7.85 (m, 2 H, H-7 and H-8), 8.25 (m, 1 H, H-9), 8.50 (m, 1 H, H-6). The infrared spectrum (KBr) showed no absorption at 2222 $cm^{-1}$. Anal. ($C_{12}H_{10}N_4O$·0.75 $H_2O$) C, H, N.

**1-Methylpyrido[2,3-*b*]quinoxalin-2(1*H*)-imine (6a).** To an ice-cooled solution of 1.0 g (4.17 mmol) of **3a** in 400 mL of tetrahydrofuran was added dropwise, over 15 min, 20 mL of an aqueous solution (20%) of titanium trichloride.[2c] After an additional 15 min, the resulting suspension was made slightly basic with saturated sodium bicarbonate solution and extracted with chlorodform. The extracts were dried (magnesium sulfate) and evaporated under reduced pressure to give an orange solid. Recrystallization from acetonitrile gave 300 mg (32%) of **6a**, mp 157–159 °C. Anal. ($C_{12}H_{10}N_4$·$H_2O$) C, H, N.

**1-Methylpyrido[2,3-*b*]quinoxalin-2(1*H*)-one 5-Oxide (7a).** A solution containing 1.0 g (4.17 mmol) of **3a** and 0.167 g (4.17 mmol) of sodium hydroxide in 400 mL of aqueous ethanol (50%) was heated at reflux for 2 h. The solution was concentrated to 50 mL, and the crude product was isolated by filtration. Re-

(7) H. L. Howes, Jr., J. E. Lynch, and J. L. Kivlin, *Antimicrob. Agents Chemother.*, 261 (1969).

(8) C. H. Issidorides and M. J. Haddadin, British Patent 1 215 815 (1970); *Chem. Abstr.*, **74**, 141873*b* (1971).

(9) A tenfold molar excess of nongaseous amines was used; reaction times varied between 16 and 72 h at 20 °C.

crystallization from aqueous ethanol (50%) afforded 650 mg (69%) of **7a**, mp 247-248 °C. Anal. ($C_{12}H_9N_3O_2$) C, H, N.

**1-Methylpyrido[2,3-*b*]quinoxalin-2(1*H*)-one (8).** A solution containing 381 mg (1.67 mmol) of **6a** and 134 mg (3.35 mmol) of sodium hydroxide in 40 mL of aqueous ethanol (50%) was refluxed 3 h. The solvent was concentrated under reduced pressure to 15 mL, diluted with water, and extracted with chloroform. The organic layer was dried (magnesium sulfate) and evaporated to a yellow solid. Recrystallization from methanol gave 160 mg (45%) of **8**, mp 243-245 °C. Anal. ($C_{12}H_9N_3O$) C, H, N.

Alternatively, **8** was prepared from **7a** by reduction with aqueous titanium trichloride as described for compound **6a**. The compound prepared in this fashion was identical in all respects with that obtained by base hydrolysis of **6a**.

**[3-(Dimethylamino)quinoxalin-2-yl]acrylonitrile 5-Oxide (9b).** A solution containing 500 mg (2.35 mmol) of either **2a** or **2b** and dimethylamine (23 mmol) in 125 mL of anhydrous methanol was stirred 16 h at 20 °C. The resulting suspension was filtered to give 337 mg (60%) of a red solid, mp 222-226 °C. The crude product was recrystallized from methanol to give 195 mg (35%) of **9b**,[10] mp 224-226 °C. The NMR spectrum ($CDCl_3$) had absorptions at δ 3.10 [s, 6 H, $N(CH_3)_2$], 5.55 (d, 1 H, $J$ = 12 Hz, CH=C*H*CN), 7.35-8.75 (m, 4 H, H-5, H-6, H-7, and H-8), 9.85 (d, 1 H, $J$ = 12 Hz, C*H*=CHCN). The infrared spectrum had a nitrile absorption at 2222 $cm^{-1}$. Anal. ($C_{13}H_{12}N_4O$) C, H, N.

**(3-*N*-Pyrrolidinequinoxalin-2-yl)acrylonitrile 5-Oxide (9c).** This compound was prepared according to the method described for **10a**. The crude product was purified by trituration with ether/chloroform (10:1) to give **9c**[10] (60%), mp 215-216 °C. Anal. ($C_{15}H_{14}N_4O$) C, H, N.

**Acknowledgment.** We thank Dr. James W. McFarland for stimulating discussions. We are indebted to Alan Fine, Larry Pisko, John Kivlin, and Jan Watrous for technical assistance.

(10) The olefinic coupling constant ($J$ = 12 Hz) is consistent with cis stereochemistry. However, a definitive assignment cannot be made without comparison to the corresponding trans isomer, which could not be isolated from the complex mixture of products in the mother liquors.

# Computer-Assisted Studies of Structure-Activity Relationships of *N*-Nitroso Compounds Using Pattern Recognition

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*Received October 26, 1981*

Pattern-recognition techniques have been applied to the study of relationships between the molecular structure of nitrosamines and their carcinogenic potential. A set of 150 nitrosamines (112 carcinogenic and 38 noncarcinogenic) was used. Each compound was represented by a set of calculated molecular structure descriptors. Discriminants were found that could separate 146 of the compounds into the two activity classes based on a set of 22 descriptors. Internal consistency checking showed that the 22 descriptors used supported a meaningful discriminant. The results show that sufficient information is contained within the structures of *N*-nitroso compounds to allow classification into carcinogenic activity classes.

An abundance of research has been reported on the carcinogenic potential of *N*-nitroso compounds in recent years. Many compounds have been synthesized and tested for carcinogenic activity by repeated administration of small doses to animals. Most of these tests are long term and expensive. The tests have led to the following observations: (1) *N*-nitroso compounds are easily formed, (2) *N*-nitroso compounds have shown activity in a wide variety of species examined, (3) *N*-nitroso compounds are active even in small doses, (4) *N*-nitroso compounds are active through a variety of administrative routes, and finally, (5) *N*-nitroso compounds show organ-specific activity.[1-3]

Hecht and co-workers[4,5] have studied tobacco-specific nitrosamines. Lijinsky[6] has performed many studies of *N*-nitroso compounds. Some cross species testing has indicated that species differences and dose rates are critical in assessing susceptibility but that all animal species are susceptible.[7] Some research has focused on nitrosamine formation by reactions of nitrites and amines[8] or through reactions of drugs with nitrites.[9,10]

Relationships between the molecular structure of *N*-nitroso compounds, their metabolism, and their carcinogenic potential have been studied extensively. Nitrosamines are thought to undergo metabolic activation to alkylating agents. The α carbon, adjacent to the *N*-nitroso group, has attracted the interest of investigators. The hydrogen atoms bonded to the α carbon have been studied using a variety of deuterated compounds to take advantage of the kinetic isotope effect. The results of these experiments have been confusing and contradictory and highlight organ specificity as a complicating factor.[11] The existence of competing metabolic pathways could explain the observations.[12,13] Studies focusing on metabolism of

(1) Lijinsky, W. In "New Concepts in Safety Evaluations"; Mahlman, M.; Shapirio, R.; Blumanbel, H., Ed.; Halsted Press: New York, 1979; Chapter 9.
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(3) Gronon, X. *Chem. Biol. Interact.* **1980**, *29*, 1-30.
(4) Hecht, S. S.; Chen, C. B.; Hoffmann, D. *Acc. Chem. Res.* **1979**, *12*, 92-98.
(5) Hecht, S. S.; Chen, C. B.; Ohmori, T.; Hoffmann, D. *Cancer Res.* **1980**, *40*, 298-302.
(6) Cardy, R. H.; Lijinsky, W.; Hildebrandt, P. K. *Ecotoxicol. Environ. Saf.* **1979**, *3*, 29-35.
(7) Maduagwu, E. N.; Bassir, O. *Toxicol. Appl. Pharmacol.* **1980**, *53*, 211-219.
(8) Walters, C. L.; Carr, F. P. A.; Dyke, C. S.; Saxby, M. J.; Smith, P. L. R. *Food Cosmet. Toxicol.* **1979**, *17*, 473-479.
(9) Lijinsky, W,; Reuber, M. D. *Food Cosmet. Toxicol.* **1980**, *18*, 85-87.
(10) Raisfeld, I. H.; Lin, C. *Biochem. Pharmacol.* **1979**, *28*, 3451-3455.
(11) Lijinsky, W. unpublished work.
(12) Lijinsky, W.; Reuber, M. D. *Cancer Res.* **1980**, *40*, 19-21.

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