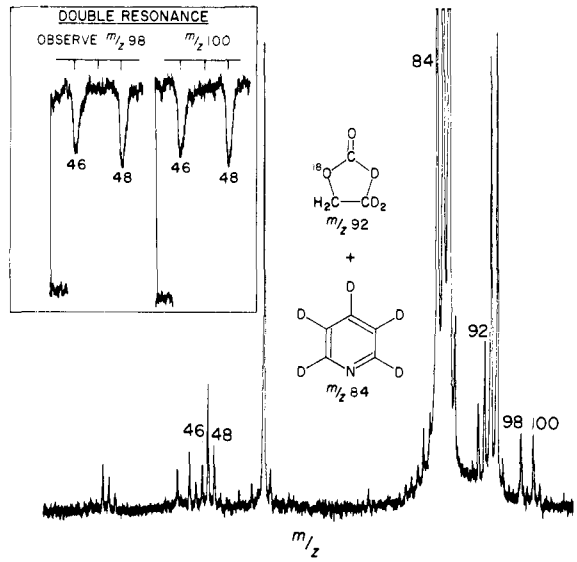

Figure 2. ICR spectrum of a mixture of 1,3-[1-^{18}O,4,4-$^{2}H_2$]dioxolan-2-one and pyridine-d_5.

would lead to the same product ions m/z 98 and 100, each of which would now have both ions, m/z 46 (**2a**) and m/z 48 (**2b**), as precursors.[10]

1,3-[1-^{18}O,4,4-$^{2}H_2$]Dioxolan-2-one was synthesized starting from ethyl diazoacetate as outlined in Scheme II, adapting a published procedure[11] to produce maximum incorporation of the ^{18}O label. The mass spectra of the doubly labeled ethylene glycol and the carbonate confirmed the incorporation (86%, ^{18}O >95% ^{2}H) and location of the labels.

The ICR spectrum[6] of a mixture of 1,3-[1-^{18}O,4,4-$^{2}H_2$]dioxolan-2-one (1.2×10^{-5} torr) and pyridine-d_5[8] (0.3×10^{-5} torr) is shown in Figure 2. Two product ions are observed at m/z 98 and 100 as a result of the transfer of $CH_2^{+\cdot}$ and $CD_2^{+\cdot}$[7] to neutral pyridine-d_5. Double resonance reveals that *each of the two product ions is formed from both precursor ions*, namely, m/z 46 and 48. This result can be explained only by assuming that the primary fragment ions have undergone a rearrangement which, at one stage, has rendered the two methylene groups equivalent. Isomers **1** and **2** are the only $C_2H_4O^{+\cdot}$ ions with equivalent methylene groups. Since **1** has been shown[3] to ring open to **2** prior to the $CH_2^{+\cdot}$ transfer reaction we can conclude that if the $C_2H_4O^{+\cdot}$ isomer **3** is generated from ethylene carbonate[9] it is not stable but undergoes an isomerization yielding, at least in part,[12] isomer **2** (Scheme I). This result is in agreement with the ab initio predictions[1] of the stabilities of the three $C_2H_4O^{+\cdot}$ isomers involved.

Acknowledgment. We thank Dr Leo Radom for his interest in this work.

(9) A referee has pointed out that the $CH_2CH_2O^{+\cdot}$ ion from ethylene carbonate may not even have a transitory existence, but that the cyclic ion **1** may be formed directly, e.g.,

$$\overline{OCOOCH_2CH_2^{+\cdot}} \rightarrow \cdot OCOOCH_2CH_2^{+} \xrightarrow{-CO_2} \mathbf{1} \rightarrow \mathbf{2}$$

or

$$\overline{COOCH_2CH_2O^{+\cdot}} \rightarrow \cdot COOCH_2CH_2O^{+} \xrightarrow{-CO_2} \mathbf{1} \rightarrow \mathbf{2}$$

On the available evidence, we cannot rule out this possibility.

(10) A similar approach has been used (Gross, M. L. *J. Am. Chem. Soc.* **1972**, *94*, 3744-3748) to establish that all three methylene groups in the $C_3H_6^{+\cdot}$ ion from tetrahydrofuran, which transfers $CH_2^{+\cdot}$ to NH_3, are identical.

(11) Paulissen, R.; Reimlinger, H.; Hayez, E.; Hubert, A. J.; Teyssie, Ph. *Tetrahedron Lett.* **1973**, 2233-2236.

(12) Our results do not exclude the possibility that **3** may in part also isomerize to other more stable $C_2H_4O^{+\cdot}$ isomers, e.g., ionized acetaldehyde.[5] Using selective ion/molecule reactions[13] we have, however, not been able to detect any evidence for the isomerization of **3** to $CH_2{=}CH{-}OH^{+\cdot}$ ions.

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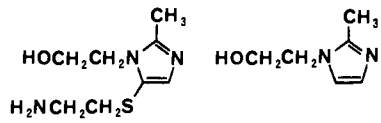
Reactions of Nitroimidazoles. Nucleophilic Substitution of the Nitro Group

P. Goldman* and James D. Wuest*,†

Department of Pharmacology, Harvard Medical School Boston, Massachusetts 02215
Received February 25, 1981

Many heteroaromatic compounds containing a nitro group have antimicrobial properties.[1] One representative of this important class of drugs is 2-methyl-5-nitroimidazole-1-ethanol (metronidazole, **1**), which is clinically effective against trichomoniasis, various forms of amoebiasis, and infections with anaerobic bacteria.[2] Despite the pharmacological importance of the nitroimidazoles, the mechanism of their metabolism and the reason for their selective toxicity toward anaerobic microorganisms have not been determined. Even the chemical behavior of this class of compounds has not been investigated thoroughly. One widely cited mechanism of biological action involves their reduction to radical anions, nitrosoimidazoles or hydroxylaminoimidazoles, followed by interaction of these hypothetical reactive metabolites with cellular macromolecules such as DNA.[3] We report here a series of reactions which emphasize for the first time the susceptibility of these simple nitro heteroaromatic compounds to nucleophilic substitution of the nitro group[4,5] and suggest that this reaction may be the basis for their biological action.



Nitroimidazole **1** reacted readily with 2-aminoethanethiol, which may be considered analogous to the biologically important nucleophile glutathione since both contain a primary sulfhydryl group.[6] At an initial pH of 5.0, the reaction of compound **1** with excess thiol under N_2 (H_2O, 37 °C, 120 h or 100 °C, 1.5 h) produced 4-[(2-aminoethyl)thio]-2-methylimidazole-1-ethanol (**2**, 98%).[7] At pH 9.5 (37 °C, 24 h) the reaction with excess thiol was significantly faster and slightly more complex, leading to compound **2** (45%) as well as to an isomeric thioimidazole, 5-[(2-aminoethyl)thio]-2-methylimidazole-1-ethanol (**3**, 22%).[7] At

†Dépertement de chimie, Université de Montréal, Montréal, Québec, Canada H3C 3V1.

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(6) (a) Willson and Searle observed that cysteine and nitroimidazole **1** react under certain conditions, but they did not identify the products of this reaction. See: Willson, R. L.; Searle, A. J. F. *Nature (London)* **1975**, *255*, 498. (b) Under other conditions, however, Boyland and Speyer report that compound **1** does not react with glutathione. See: Boyland, E.; Speyer, B. E. *Biochem. J.* **1970**, *119*, 463. (c) For a review of the chemistry of glutathione, see: Kosower, N. S.; Kosower, E. M. *Int. Rev. Cytol.* **1978**, *54*, 109.

(7) The assigned structure was consistent with the elemental analysis and the IR, NMR, and mass spectra of this new substance.

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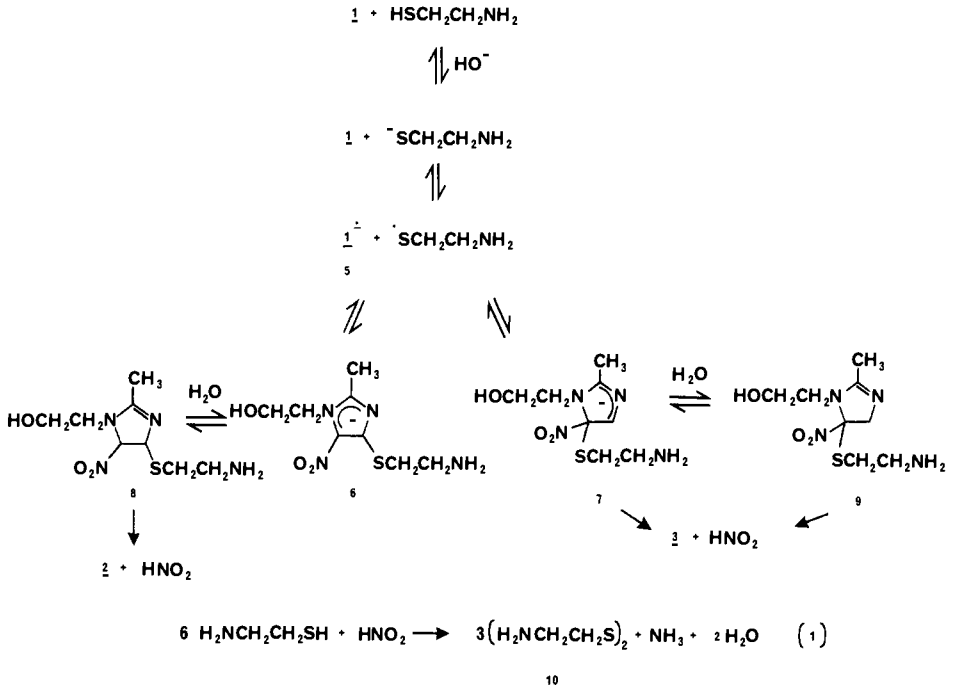
Scheme I



this higher pH, nitroimidazole **1** is also consumed by direct hydrolysis, which accounts for the reduced yield of thioimidazoles.[8,9] Assignment of structures **2** and **3** to the products is consistent with several observations. Reduction of both compounds in the presence of Raney nickel produced 2-methylimidazole-1-ethanol (**4**)[10,11] and confirmed that the compounds were thioimidazoles. In addition, their ^1H NMR spectra showed subtle differences characteristic of isomeric imidazoles.[8] H_4 in compound **3** was more shielded in D_2O than H_5 in compound **2** (δ 7.08 vs. 7.17) but less shielded in $CDCl_3$ (δ 7.01 vs. 6.88). Also characteristic was the fact that exchange of H_5 in thioimidazole **2** with deuterium (1 N NaOD, 100 °C, 20 h) was much more rapid than exchange of H_4 in thioimidazole **3**.[8]

We propose that in base these thioimidazoles are formed by the concurrent normal and abnormal substitution reactions described in Scheme I.[12,13] This sequence accounts for the accelerating effect of base and implicates the known radical anion **5** of metronidazole[14] and the reasonable Meisenheimer complexes **6** and **7**.[15] We attribute the different distribution of products at lower pH to the altered position of equilibrium between compound **1** and its conjugate acid, which might react differently with nucleophiles.[16] Indirect evidence for the elimination of nitrite was provided by the isolation of 2,2′-dithiobis[(ethanamine)] (**10**, 78%), presumably formed when nitrous acid is reduced to ammonia by excess 2-aminoethanethiol (eq 1). In fact, when typical reaction mixtures were made basic and steam distilled into aqueous hydrogen chloride, ammonium chloride could be isolated in 81% yield. More direct evidence for the elimination of nitrite came from the following experiment. When an aqueous, equimolar mixture of nitroimidazole **1** and 2-aminoethanethiol was heated (100 °C, 2 h), treated with excess *o*-phenylenediamine, and acidified with aqueous hydrogen chloride, benzotriazole was formed by diazotization in 33% yield. This low yield was not surprising since the reactions of equimolar mixtures of nitroimidazole **1** and 2-aminoethanethiol in either acid or base gave diminished yields of thioimidazoles (31% at pH 5.0, 41% at pH 8.8). This suggests that substitution of the nitro group and reduction of nitrite occur at similar rates.

Nitrous acid liberated in this way does not appear to be responsible for the lethal effect of metronidazole (**1**) on *Bacillus fragilis* and *Clostridium perfringens* since nitrite and isoamyl nitrite were significantly less toxic on a molar basis than nitroimidazole **1**.[17] We cannot exclude the possibility that compound **1** serves as a carrier of masked nitrite which is released at sensitive sites when the nitroimidazole reacts with a suitable nucleophile.

The toxicities and mutagenicities of 2-, 4-, and 5-nitroimidazoles increase in the order 4-nitro ≪ 5-nitro < 2-nitro.[18] In a series of reactions analyzed by UV spectroscopy or direct isolation of products, we compared the rates at which excess 2-aminoethanethiol reacts under N_2 (H_2O, 37 °C) with three representative nitroimidazoles: 2-nitroimidazole-1-(2-methoxymethyl)ethanol (**11**, misonidazole), compound **1**, and the isomeric compound 2-methyl-4-nitroimidazole-1-ethanol (**13**). At pH 9.5 the rates of reaction increase in the order of increasing toxicity.[19] At pH 4.8 the results are similar, and the reaction of nitroimidazole **11** with excess thiol (N_2, H_2O, 100 °C, 1.5 h) yielded 2-[(2-aminoethyl)thio]imidazole-1-(2-methoxymethyl)ethanol (**12**, 89%).[7] Under these conditions, however, compound **13** could be recovered unchanged. More vigorous conditions (H_2O, 160 °C, 17 h) were required to form the expected product of substitution, thioimidazole **3** (64%). We suggest that nitroimidazole **1** is more reactive than isomer **13** because polarization of the carbon–nitrogen double bond makes Meisenheimer complex **14** more stable than structure **15**.

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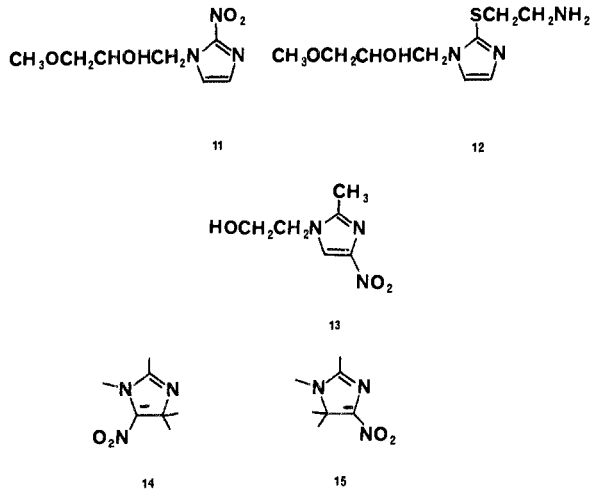
(19) Some direct hydrolysis occurs under these conditions, so the rates are not a simple measure of reactivity toward thiol.



When either bacteria or xanthine oxidase are incubated with metronidazole (**1**) under anaerobic conditions, extensive fragmentation of the imidazole ring occurs.[20] This fragmentation probably does not involve thioimidazoles as intermediates, since compound **2** is inert to aqueous acid and base and even to aqueous mercuric chloride.[21] However, preliminary experiments with water and hydrazine[9] suggest that adducts formed when metronidazole reacts with other nucleophiles may be less resistant to fragmentation. Decomposition of these adducts may lead to many of the ultimate metabolites, while conversion of other important cellular nucleophiles to inert imidazoles may account for the biological activity of nitroimidazoles.

Acknowledgment. We thank Dr. G. Jolles and Dr. J. C. Blondel of Rhône-Poulenc Santé, Paris, for a generous sample of 2-methyl-4-nitroimidazole-1-ethanol and Ed Henson for skillfully recording our mass spectra. This work was supported in part by Grant R01-CA 15,260 from the National Institutes of Health.

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Cryochemical Studies. 1. ESR Spectrum of Ag_3[1]

J. A. Howard* and K. F. Preston

Division of Chemistry, National Research Council of Canada Ottawa, Canada K1A 0R9

B. Mile

Department of Chemistry and Biochemistry Liverpool Polytechnic, Liverpool, England L3 3AF

Received May 13, 1981

There have been several ESR investigations of silver atoms isolated in rare-gas matrices at low temperatures.[2-5] Kasai and McLeod[2] found that EPR spectra in Ne, Ar, Kr, and Xe consist of two doublets of almost equal intensity from the two isotopes of silver with hyperfine interactions (hfi) up to 6% larger than the values found in the gas phase.[6] These workers also found that silver atoms react with ethylene and acetylene in rare-gas matrices to give a variety of metal atom–organic ligand complexes and pseudocomplexes.[3,4] For instance, Ag atoms and C_2H_4 give

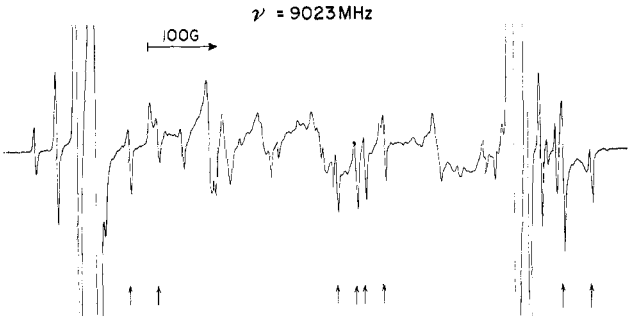

Figure 1. EPR spectrum of C_6D_6 containing ^{107}Ag at 103 K. The arrows indicate the resonance positions of Ag_3.

EPR spectra which were attributed to isolated Ag atoms, a pseudocomplex, Ag···C_2H_4, with a Ag hfi ~6% smaller than that of the free atom, and a complex, $Ag(C_2H_4)_2$, with a small Ag hfi of ~16.4 G. Ag atoms and C_2H_2 behaved somewhat differently, and EPR spectra have been assigned to the pseudocomplexes Ag···C_2H_2, Ag···$(C_2H_2)_2$, Ag···$(C_2H_2)_{n\geq 3}$, and the vinyl radical AgCH=CH. Recently, Ozin[5] using high concentrations of silver in Ar and Kr ($1:10^2$–$1:10^3$) and photoaggregation of matrices dilute in silver ($1:10^3$–$1:10^4$) obtained spectra which were weak in Ag atoms and strong in an isotropic feature centered at $g \sim 2$. Ozin concluded that the latter spectrum was a composite of two spectra, one consisting of sharp lines which were attributed to a range of silver aggregates with molecular cluster properties and a broader conduction EPR spectrum which was assigned to small silver microcrystallites.

We wish to report here the first positive EPR identification of a neutral silver cluster (Ag_3) which has been produced at 77 K by cocondensation of ^{107}Ag atoms and C_6D_6 on the cold surface of a rotating cryostat.[7,8] Isotopically pure silver (98.22% ^{107}Ag from Oak Ridge National Laboratory, TN) was chosen for these experiments because of the anticipated complexity of the spectrum from natural silver. The EPR spectrum obtained from this experiment is shown in Figure 1. It is dominated by two doublets with large isotropic hyperfine splitting constants (a_{107}^{I} = 608.2 G, g_{iso} = 2.0004; a_{107}^{II} = 562.45 G, g_{iso} = 1.9926) which are associated with isolated atoms and the pseudocomplex Ag···C_6D_6.[9]

A more complex spectrum, again essentially isotropic, consists of four ~40-G doublets (arrows in Figure 1). The separation of the central pair of doublets is precisely that expected for a second-order splitting[10] associated with equal (~295 G) isotropic hyperfine interactions from two nuclear spins of magnitude $^1/_2$. We assign the spectrum to the cluster of three silver atoms Ag_3 in which the equivalent terminal nuclei show equal, larger hyperfine interactions.

An exact least-squares solution of an isotropic spin Hamiltonian for Ag_3 using all eight lines of the observed spectrum led to the following best-fit parameters: $a_{107}(2)$ = 295.0 ± 0.3 G, $a_{107}(1)$ = 38.5 ± 0.3 G, and g_{iso} = 1.9622 ± 0.0001. Closer inspection of the spectrum at high resolution suggested the presence of residual g anisotropy from an orthorhombic tensor with principal values ~1.960, 1.962, and 1.966. There was, however, no suggestion of ^{107}Ag hyperfine anisotropy.

Using the appropriate one-electron parameter for ^{107}Ag,[11] the isotropic hyperfine interactions in Ag_3 may be converted to spin populations of 44% for each of the terminal Ag(5s) and 6% for the central Ag(5s) atomic orbital. The composition of the semioccupied orbital (SOMO) of Ag_3 is thus strikingly similar to those of the alkali-atom clusters Na_3[12] and K_3.[13] In all three cases, the unpaired electron is more or less localized in valence s atomic

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