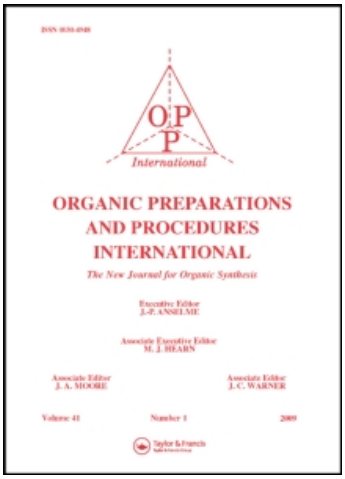

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ADDUCTS OF PIPERAZINE WITH NITRIC OXIDE

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ADDUCTS OF PIPERAZINE WITH NITRIC OXIDE

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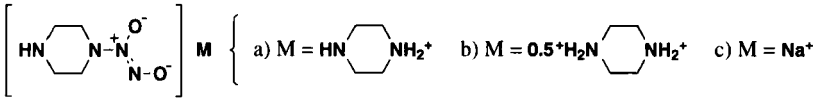
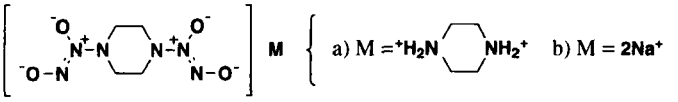
Our efforts to characterize the biomedical utility of the diazeniumdiolates (compounds containing the [N(O)NO]$^-$ functional group)[1] have been greatly facilitated by the favorable properties of the polyamine/NO adducts.[2] Unfortunately, most linear diamines and triamines have no current pharmaceutical use and thus their formation as the initial by-products of NO donation by these compounds could lead to unanticipated, possibly undesirable pharmacological consequences. Since these concerns do not apply to the cyclic diamine piperazine, which has been used extensively as an anthelmintic,[3] we wished to prepare anionic piperazine/NO adduct **1** for tests of its pharmacological activity. There exist two previous accounts of the reaction between piperazine and NO, both of which



1

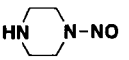
greatly predate our own extensive polyamine studies. One report[4] concerns the preparation of *bis*-diazeniumdiolates **2a** and **2b**. More noteworthy in the present context is the preparation of the monodiazeniumdiolate **1** as the monoprotonated piperazinium salt (**1a**) by Reilly[5] using a high pressure (24 atm NO) reaction in an autoclave. We have reinvestigated this reaction and have developed a convenient low pressure (5 atm) preparation of two new, more useful salts of anion **1**. This report describes our synthesis of piperazine monodiazeniumdiolates **1b** and **1c** and the physicochemical profiles of compounds **1** and **2**.



2

Reilly reported isolating a 50% yield of **1a** by heating a 1:1 (w:w) mixture of piperazine with methanol at 30° for 7.5 h under 24 atm of NO and then precipitating the product with acetone. We repeated this reaction and found that the product contained two different piperazine rings in equal abundance [^{1}H NMR in D_2O/NaOD δ 3.10 (m, 4H); 3.04 (m, 4H); 2.81 (s, 8H)], consistent with its formulation as the 1:1 salt **1a** (We take both the NMR and UV spectra in basic solution because the diazeniumdiolates decompose at neutral or lower pH. Please note that this means that we actually obtain the spectra of the anion plus, if applicable, the liberated piperazine.). This effectively represents a 25% yield of the desired active NO donor and direct pharmaceutical utilization of this material would result in a large collateral dose of piperazine. Our attempts to convert this material to the sodium salt of anion **1** were complicated by the presence of this large quantity of piperazine which tended to coprecipitate. Fortunately, we discovered that by using a relatively dilute solution of the amine in acetonitrile in an unheated reaction with NO it was possible to isolate a termolecular salt which contained two anions **1** and one diprotonated piperazinium dication in high yield. This salt exhibited a proton spectrum consistent with structure **1b** and this formulation was confirmed by elemental analysis. Further, the solubility properties of this salt enabled us to perform a simple metathesis with sodium methoxide leading to the isolation of the sodium salt of the piperazine monodiazeniumdiolate (**1c**) in pure form. This material displays the expected AA'BB' proton NMR pattern for the two groups of four equivalent protons. We have previously noted[2] the role of solubility in influencing the outcome of reactions of amines with NO and this appears to be a particularly striking example which permits the synthetic chemist to select from among three possible outcomes (formation of **1a** in hot methanol,[5] **1b** in acetonitrile, or **2a** in cold methanol[4]) merely by changing the solvent.

bis-Diazeniumdiolate **2b** (dihydrate, formula weight 286) was prepared from **2a** *via* a metathesis with sodium methoxide by the method of Longhi *et al.*[4] Since there is free rotation about the N-N bond joining the [N(O)NO]$^-$ groups to the ring, the proton spectrum of **2b** consists of a singlet at δ 3.2. There was no evidence for the presence of **2** in any of the preparations of **1a-c** and the cleanliness of the proton NMR spectra suggests that neither N-mono-(**3**) nor N,N'-*bis*-nitrosopiperazine (**4**) is present in these samples.



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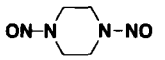

4

The ultraviolet spectra of these compounds were similar to those of other simple diazeniumdiolates,[1] showing single maxima in dilute alkali at 252 nm for both **1** (ε = 7.8 $mM^{-1}cm^{-1}$) and **2** (ε = 17.6 $mM^{-1}cm^{-1}$).

Compounds **1** and **2**, regardless of the nature of the cation, resembled other simple diazeniumdiolates in their ability to regenerate NO spontaneously on dissolution in aqueous media,[1] releasing an observed 2.1 and 4.3 mol of NO, respectively, in pH 7.4 phosphate buffer. First-order dissociation of **1** occurred with a half-life of 5.3 min ($k = 2.2 \times 10^{-3}$ s^{-1}) at 37° under these conditions. For **2**, first-order plots did not display good linearity, but showed evidence of biphasic kinetics. Data were fitted to a reaction scheme involving two successive first-order reactions, an initial change ($k_1 = 4.9 \times 10^{-3}$ s^{-1}, half-life 2.3 min) followed by a slower second step; the rate constant for the latter ($k_2 = 2.3 \times 10^{-3}$ s^{-1}) compares well with that reported above for **1**, as expected for the two-stage dissociation of diazeniumdiolate groups in **2**.

NO release from both **1** and **2** was accompanied by surprisingly extensive formation of both **3** and **4**. Under aerobic conditions as a 7.0 mM solution, **1** produced a 16% yield of **3** and even more **4** (18%), while **2** produced a much larger yield of **4**. When the reaction was repeated using argon to sweep the liberated NO from the solution as it formed, the yield of **4** from **1** was reduced to 12% and this was further reduced to 3.9% by a 10-fold decrease in the concentration of **1**. Compound **4** is a potent carcinogen in experimental animals.[6] Its production as a by-product of NO generation from the diazeniumdiolated piperazines demands that considerable caution be used in contemplating their potential clinical utility. Since lowering the initial concentration of **1** decreased the yield of **4**, it is possible that production of the carcinogen would be suppressed entirely in the limit of the low doses of **1** required to yield the quantities of NO needed for some pharmacological applications. Generation of **3** by a non-dissociative mechanism might still be envisioned, but **3** has been judged to be non- or weakly carcinogenic,[6] so its appearance as a metabolite or decomposition product of **1** may be of lesser concern toxicologically. The ability of **1** to stimulate erections in male cats[7] suggests its potential utility as an NO donor prodrug if exhaustive toxicity profiling shows it to pose an acceptable risk. The possibility that **2** could be transformed to **4** even at infinite dilution militates against its use in any clinical setting, although the high latent NO content of **2** could be of considerable benefit as a tool for probing the biological properties of NO in certain research applications.

EXPERIMENTAL SECTION

Proton NMR spectra were recorded at 200 MHz and carbon NMR spectra were recorded at 50 MHz, both on a Varian XL-200. Ultraviolet (UV) spectra were obtained using a Hewlett-Packard Model 8451A diode array spectrophotometer. Chemiluminescence measurements of NO were performed with Thermal Energy Analyzer Model 502A from Thermedics, Inc. (Woburn, MA). Elemental analyses were obtained from Atlantic Microlab, Inc. (Norcross, GA). Melting points are uncorrected.

Preparation of 1b.- A solution of 5.0 g anhydrous piperazine (58 mmol) in 200 mL acetonitrile was stirred under 80 psig NO as described previously.[2] After 16 h, the colorless precipitate was isolated by filtration, washed with acetonitrile, then ether and dried under vacuum. Yield 6.6 g (90%); mp 106-107° dec; ^{1}H NMR (D_2O + NaOD): δ 2.72 (8H, s), 2.94-3.00 (8H, m), 3.05-3.11 (8H, m); ^{13}C NMR (D_2O + NaOD): δ 45.5, 46.2 (cation C's), 53.6; UV λ_{max} (0.01 M NaOH) 252 nm ($\varepsilon = 7.85$ mM^{-1} cm^{-1}).

Anal. Calcd. for $C_{12}H_{30}N_{10}O_4$: C, 38.09; H, 7.99; N, 37.01. Found: C, 38.15; H, 8.00; N, 37.05

Preparation of 1c.- A solution of NaOMe in MeOH was prepared by diluting 4.00 g of 25% NaOMe in MeOH (18.5 mmol NaOMe) with 4 mL MeOH and to this was added with stirring 3.50 g **1b** (9.25 mmol). After complete dissolution, the colorless sodium salt was precipitated by slow addition of 10 mL acetonitrile and then isolated by filtration, washed with ether and dried under vacuum. Yield 2.74 g (88%); mp >200° (chars). ^{1}H NMR (D_2O): δ 2.86-3.02 (4H, m), 3.08-3.13 (4H, m); ^{13}C NMR (D_2O): δ 45.5, 53.6; UV λ_{max} (0.01 M NaOH) 252 nm (ε = 7.85 mM^{-1} cm^{-1}).

Anal. Calcd. for $C_4H_9N_4O_2Na \cdot 1/3H_2O$: C, 27.61; H, 5.51; N, 32.20; Na, 13.21

Found: C, 27.83; H, 5.55; N, 32.19; Na, 13.29

NO Release Studies.- Quantities of NO released per mole of **1** and **2** in pH 7.4 phosphate buffer were measured by chemiluminescence as previously described.[1] Rate measurements were carried out spectrophotometrically by following the decrease in the UV absorbance maximum at 252 nm. Measurements were made in 0.10 M phosphate buffer at 37°.

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