N-Nitrosated *N*-hydroxyguanidines are nitric oxide-releasing diazeniumdiolates

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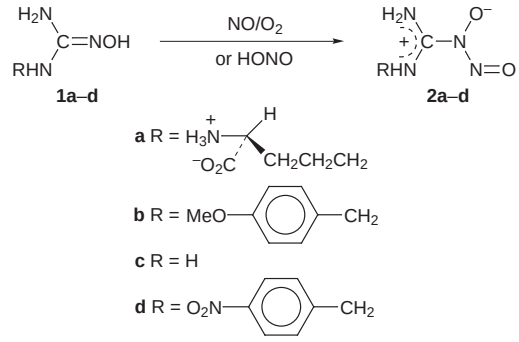
***N*-Hydroxyguanidines can be nitrosatively converted to zwitterionic diazeniumdiolates of crystallographically-confirmed structure H_2N^+=C[NHR][N(O)NO]$^-$, whose hydrolytic dissociation at physiological pH leads to both NO and N_2O; the results appear to account for the formation of the 'potential intercellular nitric oxide carrier' produced on exposing N^G- hydroxy-L-arginine (a metabolic intermediate in mammalian NO biosynthesis) to aerobic NO.**

Nitric oxide (NO) is a recently discovered bioeffector molecule that is critically involved in regulation of blood pressure, neurotransmission, sexual function, immunity and an array of other physiological phenomena.[1] It is rapidly destroyed by biochemical oxidants such as oxyhemoglobin, superoxide and oxygen,[2] leading to speculation that its regulatory properties might depend, in part, on conversion to a biochemical storage form[3] that protects it from oxidation while in transit from the site of biosynthesis in one cell to the target of its required signalling action in another.

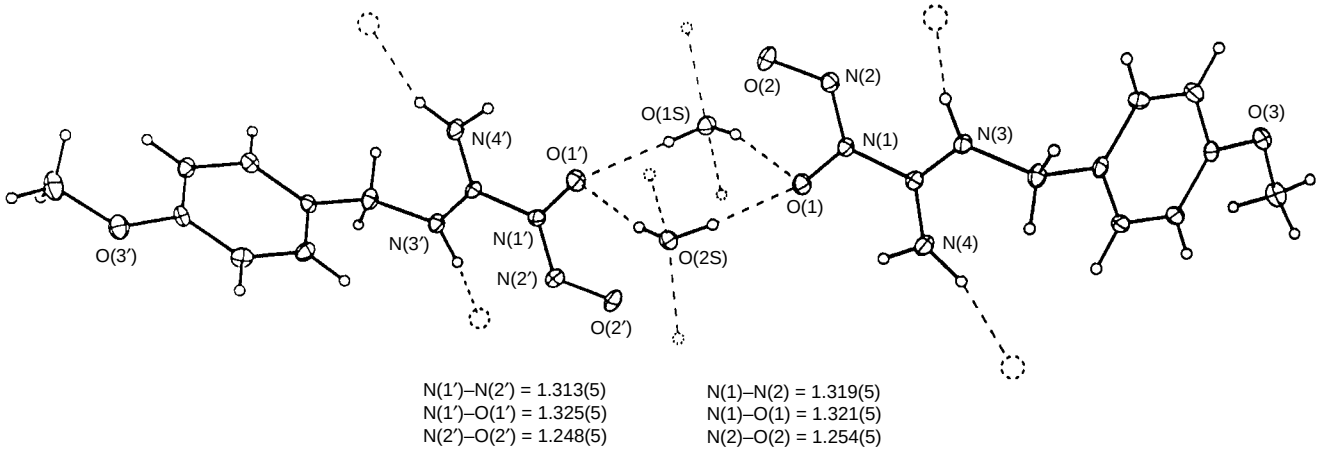
Recently, the formation of such a 'potential intercellular nitric oxide carrier' was reported to occur on exposing N^G-hydroxy-L-arginine **1a**, an intermediate in NO biosynthesis, to aerobic nitric oxide solutions.[4] The product was ultraviolet-active and longer lived as a vasodilator than molecular NO,[5] but its structure has yet to be elucidated.

We present evidence here based on work with model *N*′-substituted *N*-hydroxyguanidines that this bioactive compound may be a naturally occurring diazeniumdiolate (Scheme 1, structure **2a**). While there was no observable reaction when NO was bubbled into degassed solutions of *N*′-(*p*-methoxybenzyl)-*N*-hydroxyguanidine **1b** in water, subsequent introduction of air led to appearance of an ultraviolet maximum at 322 nm, a result reminiscent of that seen with both *N*-hydroxyguanidine **1c**[5] and **1a** itself.[6] A similar outcome was observed when NO_2 was introduced instead of air into anaerobic solutions of **1b** and NO, suggesting that N_2O_3, formed either on autoxidation of NO or on radical coupling of NO with NO_2, had reacted nitrosatively[7] with **1b**. When alternate nitrosation conditions were effected by dissolving 0.1 g of **1b**·HCl in 2 ml of 0.1 M aqueous AcOH and adding 1 equiv. of sodium nitrite as a saturated aqueous solution at 0 °C, a crystalline precipitate began to form that, when collected after 20 min of total reaction time (yield of **2b** 95%, mp 136–147 °C with gradual decomposition), was suitable for X-ray diffractometric investigation without further purification. (Indeed, attempts to recrystallize this relatively unstable substance have thus far led to partial decomposition.) The molecular structure,§ refined to an *R* value of 0.034, is shown in Fig. 1.

The crystallographic data show that nitrosation of **1b** occurred at the nitrogen bound to the oxygen atom to give a diazeniumdiolate product **2b**, as shown in Scheme 1. Noteworthy structural features include the cisoid oxygens in the



Scheme 1



N(1′)–N(2′) = 1.313(5)
N(1′)–O(1′) = 1.325(5)
N(2′)–O(2′) = 1.248(5)

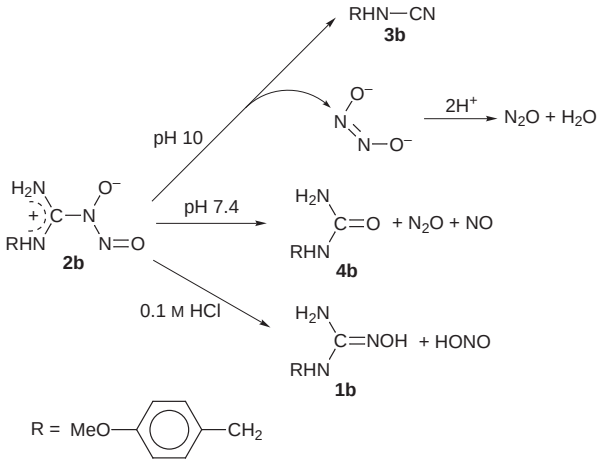
N(1)–N(2) = 1.319(5)
N(1)–O(1) = 1.321(5)
N(2)–O(2) = 1.254(5)

Fig 1 Molecular structure of the asymmetric unit of **2b** summarizing bond lengths within the diazeniumdiolate (ONNO) groups. Dotted atoms are symmetry related. Dotted bonds are hydrogen bonds.

$N_2O_2^-$ group and the presence of two different conformers of **2b** along with two water molecules in the asymmetric unit that forms a hydrogen bonded helix (Fig. 1). Similar results were obtained on nitrosation of the *p*-nitrophenyl analogue **1d**; although the structure of the diazeniumdiolate product **2d** did not refine well (R = 0.125), this was due to disorder in the crystal which could not be modelled in the refinement. The atomic connectivity and atom assignments are, however, well defined.¶

Rapid measurement of the ultraviolet spectrum on dissolution of **2b** in water permitted quantitative determination of the characteristic chromophore's extinction coefficient (2.5 mM^{-1} cm^{-1} at λ_{max} = 322 nm). The NMR spectrum, run at −50 °C in CD_3OD–tetramethylsilane to minimize the rapid decomposition observed at room temperature, consisted of singlets for the CH_3O and CH_2 protons at δ 3.77 and 4.57, respectively, and an aryl AA′BB′ pattern with shifts of δ 6.93 and 7.30 (*ortho* and *meta* to OCH_3, respectively) and J = 8.6, 2.7, and 0.4 Hz for the *ortho*, *meta* and *para* couplings, respectively. The mass spectra of **2b** and **2d** were unusual (but not unprecedented) in their failure to produce an observable MH^+ ion in either the electrospray or fast atom bombardment modes; strong peaks due to the respective benzyl cations were seen in both cases, but ^{15}N-labelling indicated that the terminal NO was rapidly lost even under the mildest possible electrospray conditions.

Compound **2b** was found to decompose with gas evolution on dissolution in aqueous media. At pH 10, *N*-(*p*-methoxybenzyl)cyanamide **3b** and N_2O were each produced in ⩾95% yield in a relatively slow reaction (half-life at 37 °C variable but estimated as 15–25 min) that is presumably initiated by deprotonation of the NH_2 group followed by loss of *cis*-hyponitrite (⁻O–N=N–O⁻), a known progenitor of N_2O.[8] Compound **2b** tended to disappear more rapidly as pH was lowered, with a half-life at pH 3 of about 5 min; some denitrosation was observed at very low pH, **1b** being the most abundant of the organic products seen in 0.1 M HCl. The major gaseous product at low pH proved to be NO, reaching yields of 0.9 moles per mole of **2b** at pH 3 compared with 0.25 moles of N_2O. While the mechanism of NO formation is not clear at this time, there can be little doubt about its production; its identity was confirmed both by the presence of its aqueous autoxidation product, nitrite ion,[9] in the reaction mixture and by a well-established, highly selective chemiluminescence method.[10] In physiological buffer (10 mM phosphate, pH 7.4), *N*-(*p*-methoxybenzyl)urea **4b** was produced in 90% yield, with 8% conversion to **3b** and a small amount of an unidentified product also detected by HPLC. At pH 7.4, 0.5 moles of N_2O were produced along with 0.3 moles of NO per mole of **2b** dissociated. These reactions are summarized in Scheme 2. Comparable results were observed on hydrolysis of **2c** and **2d**.



Scheme 2

Since nitrosation of all four *N*-hydroxyguanidines **1a–d** yields hydrolytically unstable products with ultraviolet maxima near 320 nm that generate significant quantities of NO in neutral buffer, the crystallographic data for products **2b** and **2d** strongly suggest that the 'nitric oxide carrier' seen by Hecker *et al.*[4] on exposing **1a** to aerobic NO was diazeniumdiolate **2a**.

We postulate that our data may be of two-fold biological significance. First, if free **1a** can be shown to encounter suitably nitrosating conditions *in vivo*, the product **2a** would constitute a naturally occurring diazeniumdiolate that spontaneously releases NO at physiological pH; two other natural products containing the diazeniumdiolate functional group, dopastin and alanosine, have been reported to release NO only on one-electron oxidation.[11] Second, the NO-generating properties of compounds **2** may render them useful as prodrugs for treating clinical disorders arising from deficiencies of biosynthetic NO.[1] The possible medicinal value of **2** and analogous structures will be investigated.

Notes and References

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§ *Crystal data* for **2b**. $C_9H_{12}N_4O_3{\cdot}H_2O$, M_r = 242.24, monoclinic space group $P2_1$, a = 12.452(2), b = 7.156(1), c = 12.887(2) Å, β = 97.74(1)°, V = 1137.8(3) Å^3, Z = 4, D_c = 1.414 Mg m^{-3}, λ(Mo-Kα) = 0.71073 Å, μ = 0.113 mm^{-1}, F(000) = 512, T = 223 K. A set of 1773 reflections was collected, and 1388 were observed with $F_o > 4\sigma(F_o)$, $R1$ = 0.034 and $wR2$ = 0.083.

¶ Crystals were triclinic, space group $P\bar{1}$, a = 6.256(1), b = 7.120(3), c = 13.180(2) Å, α = 95.11(1), β = 92.33(1), γ = 106.01(1)°. CCDC 182/839.

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