
***N*-Nitropyrzoles, a new source of nitrogen monoxide**

Nikita B. Grigor'ev,^a Viktoriya I. Levina,^a Svyatoslav A. Shevelev,^{*b} Igor L. Dalinger^b and Vladimir G. Granik^{*a}

^a Centre for Medicinal Chemistry, All-Russian Chemical-Pharmaceutical Institute, 119815 Moscow, Russian Federation.

Fax: +7 095 246 6633

^b N. D. Zelinsky Institute of Organic Chemistry, Russian Academy of Sciences, 117913 Moscow, Russian Federation.

Fax: +7 095 135 5328

Electrochemical reduction of *N*-nitropyrzoles, and chemical reduction by cysteine or potassium ferrocyanide, results in nitrogen monoxide.

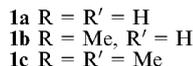
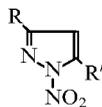
The present paper deals with the study of a new source of nitrogen monoxide – *N*-nitropyrzoles which are of considerable biological interest as new vasodilators.

It was recently shown that organic nitrates and nitrites (nitroglycerine, nitrosorbitol, amyl nitrite, *etc.*), which are extensively used as antianginal agents, cause vasculum dilation and suppress the pain syndrome due to formation of nitrogen monoxide during their biotransformation.^{1,2} The latter (called an endothelial relaxing factor) brings about the activation of the guanylate cyclase enzyme and favours the formation of guanosine-3',5'-monophosphate (c-GMP), which acts as a vasodilatation mediator. c-GMP affects the phosphorylation of intracellular peptides, the exchange of Ca²⁺ ions and other

processes effecting the relaxation of a vasculum wall. At present, NO is regarded as a very important endogenic factor in the regulation of diverse physiological processes.^{3,4} In view of this, much attention is paid in the literature to searching for new compounds capable of generating nitrogen monoxide by various mechanisms.^{5,6}

In this respect, it seemed to be of interest to study *N*-nitropyrzoles,⁷ since it is known that their electrochemical reduction (unlike that of *N*-nitroamines such as *N*-nitroaniline, *N*-nitro-2-aminopyridine, *etc.*^{8,9}) yields nitrite ions.^{10,11} Taking into account the fact that reduction of nitrates (*e.g.*, nitroglycerine) also produces NO₂⁻, which then undergoes irreversible one-electron reduction into NO,¹²

reactions (1) and (2), we studied the possibility of formation of nitrogen monoxide in the electrochemical and chemical reduction of 1-nitropyrazole **1a**, 1-nitro-3-methyl- **1b** and 1-nitro-3,5-dimethylpyrazoles **1c**.[†]



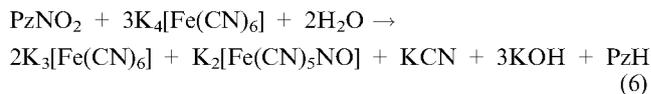
Polarograms recorded during the reduction of **1a,b** displayed, in each case, one two-electron irreversible wave with $E_{1/2} = -0.35$ V; the second wave ($E_{1/2} = -1.15$ V) corresponding to one-electron irreversible discharge of NO_2^- appeared in a solution of 5×10^{-2} M HCl + 0.2 M NaClO_4 . A somewhat different picture was observed for compound **1c**, probably because of steric interaction between the 1-nitro group and the 5-methyl substituent. Two waves were recorded on the polarograms for this compound, the first one ($E_{1/2} = 0.0$ V) not dependent on pH, whereas the other two-electron wave shifted in the positive direction as pH decreased.[‡] Similarly for compounds **1a,b**, an NO_2^- discharge wave appeared in dilute acid solutions. Thus, unlike **1a,b**, three waves can be observed for **1c**. We attribute the first wave recorded for **1c** to cleavage of the N- NO_2 bond, the second wave to the possible reduction of the nitro group,[§] and the third wave to discharge of NO_2^- .

Since $E_{1/2}$ characterises (for an irreversible process) the difference between the energies of the transition and starting states, it can be assumed that the N- NO_2 bond in compound **1c** is weaker (due to the steric effect), which can cause the easier elimination of NO_2^- , and hence NO, for this compound. It follows from literature data¹⁵ that endogenous thiols, e.g. cysteine, serve as carriers of NO to the heme of guanylate cyclase enzyme. Hence, the ability (including the comparative ability) of compounds **1a-c** to release NO was estimated by studying the reaction of these compounds with cysteine, as was done previously¹⁶ for nitroglycerine. It was found that in the absence of O_2 in 5×10^{-5} mol dm^{-3} solutions of **1a-c** with the addition (10^{-4} mol dm^{-3}) of cysteine in a citrate-borate buffer (pH 7.2), the anodic wave ($E_{1/2} = -0.46$ V) of cysteine oxidation decreased with time, in agreement with reactions (3)–(5) (see, e.g., ref. 17):



It was shown that the rate of this reaction determined from the time dependence of the limiting anodic current with respect to cysteine for compound **1c** ($k \sim 5 \times 10^{-4}$ s⁻¹) is higher than those for **1a,b** ($k \sim 10^{-4}$ s⁻¹). This corresponds to a higher oxidation ability (and lower strength of the N- NO_2 bond) of compound **1c**, in agreement with the $E_{1/2}$ values for these compounds. Even more impressive evidence of this fact is the reaction of compounds **1a-c** with potassium hexacyano-

ferrate(II) (see ref. 18 for a similar reaction of nitroglycerine). We managed to detect the formation of the nitroprusside ion, $E_{1/2} = -0.1$ and -0.6 V, by differential pulse polarography in a citrate buffer solution (pH 5), 2×10^{-4} mol dm^{-3} **1c** and 10^{-3} mol dm^{-3} $\text{K}_4[\text{Fe}(\text{CN})_6]$ under the conditions reported in ref. 18 [reaction (6)].



Unlike **1c**, nitroprusside ion formation was not observed under these conditions for **1a,b**, this being consistent with the more negative $E_{1/2}$ values for these compounds and, hence, their lower oxidising ability. Reduction of **1a,b** with potassium hexacyanoferrate(II) at lower pH values, e.g. at pH 3, yields the nitroprusside ion ($E_{1/2} = -0.25$ and -0.45 V).

Thus, the similarity revealed between the mechanisms of electrochemical and chemical reduction of *N*-nitropyrazoles **1a-c**, i.e. liberation of nitrogen monoxide, allows us to state that these compounds can serve as sources of NO and, hence, gives us the possibility of predicting the antianginal activity of compounds of this type[¶] (it should be stressed once more that the biotransformation of nitroglycerine and other nitrates occurs with the participation of cysteine). Preliminary data obtained by Professor I. S. Severina at the Institute of Medicinal and Biological Chemistry of the Russian Academy of Medical Sciences suggest that compound **1c** activates soluble guanylate cyclase, which supports the above assumption.

References

- 1 L. J. Ignarro, H. Lipton and J. C. Edwards, *J. Pharmacol. Exp. Ther.*, 1981, **218**, 739.
- 2 L. J. Ignarro, J. B. Adams, P. H. Horwitz and K. S. Wood, *J. Biol. Chem.*, 1986, **261**, 4997.
- 3 S. Moncada, R. M. J. Palmer and E. A. Higgs, *Pharmacol. Rev.*, 1991, **43**, 109.
- 4 D. S. Bredt and S. H. Snyder, *Neuron*, 1992, **8**, 8.
- 5 J. Loscalzo, D. Smick, N. Andon and J. Cooke, *J. Pharmacol. Exp. Ther.*, 1989, **249**, 726.
- 6 I. S. Severina, I. K. Ryaposova, L. B. Volodarsky, D. C. Mazhukin, A. Ya. Tichonov, G. Ya. Schwartz, V. G. Granik, D. A. Grigoryev and N. B. Grigoryev, *Biochemistry and Molecular Biology International*, 1993, **30**, 357.
- 7 J. H. Boyer, *Nitroazoles*, VCH, Weinheim, 1986.
- 8 E. Laviron, P. Fournari and J. Grensard, *Bull. Soc. Chim. Fr.*, 1967, 1255.
- 9 G. F. Wright, *Khimiya nitro- i nitrozogrupp (Chemistry of nitro and nitroso groups)*, Mir, Moscow, 1972, vol. 1, pp. 473–474 (in Russian).
- 10 E. Laviron and P. Fournari, *Bull. Soc. Chim. Fr.*, 1966, 518.
- 11 G. I. Vakul'skaya, L. I. Larina, O. B. Nefedova and V. A. Lopyrev, *Khim. Geterotsikl. Soedin.*, 1982, 523 [*Chem. Heterocycl. Compd. (Engl. Transl.)*, 1982, 400].
- 12 G. C. Whitnack, J. M. Nielson and F. S. K. Gantz, *J. Am. Chem. Soc.*, 1954, **76**, 4711.
- 13 J. W. A. M. Janssen, H. Y. Koeners, C. Gr. Kruse and C. Habraken, *J. Org. Chem.*, 1973, **38**, 1777.
- 14 S. G. Mairanovskii, Ya. P. Stradyn and V. D. Bezuglyi, in *Polyarografiya organicheskikh soedinenii (Polarography of organic compounds)*, Khimiya, Leningrad, 1975, pp. 325–347 (in Russian).
- 15 R. F. Furchgott and I. V. Zawadsky, *Nature*, 1980, **288**, 373.
- 16 N. B. Grigor'ev, G. Ya. Shvarts and D. A. Grigor'ev, *Khim. Farm. Zh.*, 1991, **5**, 12 (in Russian).
- 17 D. L. H. Williams, *Chem. Soc. Rev.*, 1985, **14**, 171.
- 18 V. I. Levina, D. A. Grigor'ev and N. B. Grigor'ev, *Khim. Farm. Zh.*, 1995, **8**, 56 (in Russian).

Received: Moscow, 26th June 1995;
Cambridge, 27th September 1995; Com. 5/04295J

[†] Compounds **1a-c** were synthesized by the method described in ref. 13; polarographic measurements were performed according to the procedure in ref. 14.

[‡] In the pH range from 0.5 to 2.0, $\Delta E/\Delta \text{pH} = -75$ mV.

[§] The mechanism of electrochemical reduction of **1c** will be thoroughly studied later.

[¶] We are planning to synthesize and study a wide range of *N*-nitropyrazoles with various substituents at the pyrazole ring as sources of nitrogen monoxide.