Pergamon

Tetrahedron Letters 41 (2000) 3637–3641

TETRAHEDRON LETTERS

Influence of structure on N–NO bond cleavage of aliphatic *N*-nitrosamines†

Motoko Miura,[a] Shigeru Sakamoto,[b] Kentaro Yamaguchi [b] and Tomohiko Ohwada [a,*]

[a] *Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori, Mizuho-ku, Nagoya 467-8603, Japan*

[b] *Chemical Analysis Center, Chiba University, Yayoi-cho, Inage-ku, Chiba 263-8522, Japan*

Received 17 February 2000; revised 9 March 2000; accepted 10 March 2000

Abstract

N-Nitrosamines can be considered as potential NO/NO^+ donors. Previous studies demonstrated that aromatic *N*-nitrosoureas and aromatic *N*-nitrosamines can act as donors of NO. The relation of the structures of *N*-nitrosamines, in particular of aliphatic *N*-nitrosamines, to the characteristics of release and capture of NO or its redox forms remains unclear. In this paper we show that aliphatic *N*-nitrosamines of 7-azabicyclo[2.2.1]heptanes can undergo N–NO bond cleavage, and we also postulate that *N*-nitrosamines which enhance N–NO bond cleavage have low rotational barriers with respect to the N–NO bonds.

Keywords: nitrosamines; bicyclic heterocyclic compounds; bonds; NMR.

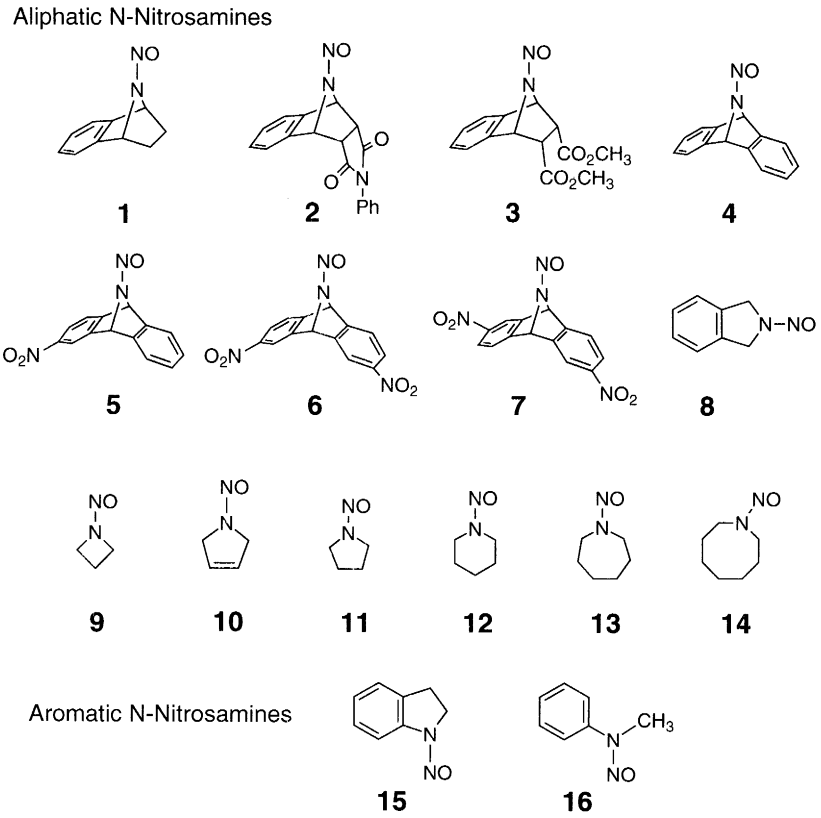
Nitric oxide (NO) has diverse roles in regulating many important physiological functions in a wide range of target tissues.[1,2] Nitrosonium ion (NO^+), a redox form of NO, is proposed to be involved in the formation of *S*-nitrosothiols and *S*-nitrosoproteins in plasma which can give NO.[3,4] The phenomena of NO/NO^+ release and NO/NO^+ capture are of interest, not least because NO/NO^+ donors are candidate drugs for vascular tone regulation in endothelium[5a,6] and for modulation of the activity of the central and peripheral nervous systems.[5b,6] *N*-Nitrosamines can be considered as potential NO/NO^+ donors,[6] although some of these compounds are known to be carcinogens and mutagens.[7–11] Previous studies demonstrated that aromatic *N*-nitrosoureas and aromatic *N*-nitrosamines can act as donors of NO.[12–14] Although many structural studies of *N*-nitrosamines, including crystallographic analysis[7] and NMR studies[15–17] have been reported, the relation of the structures of *N*-nitrosamines, in particular of aliphatic *N*-nitrosamines, to the characteristics of release and capture of NO or its redox forms remains unclear. The aims of this paper are: (1) to show that aliphatic *N*-nitrosamines of 7-azabicyclo[2.2.1]heptanes can undergo N–NO bond cleavage; and (2) to propose structural features of *N*-nitrosamines that enhance N–NO bond cleavage.

* Corresponding author. Tel/fax: +81-52-836-3407; e-mail: ohwada@phar.nagoya-cu.ac.jp (T. Ohwada)

† Dedicated to the memory of Prof. Kyosuke Tsuda.

0040-4039/00/$ - see front matter
PII: S0040-4039(00)00431-7

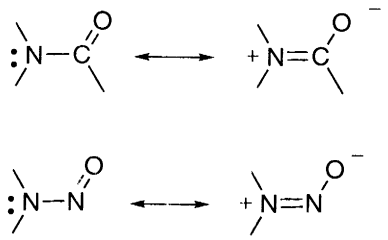
We prepared a range of monocyclic aliphatic *N*-nitrosamines and also bicyclic *N*-nitrosamines, i.e. *N*-nitroso derivatives of 7-azabicyclo[2.2.1]heptanes, in order to assess the effect of the additional bridging in these structures (Scheme 1).[18] Structually related aromatic *N*-nitrosamines were also prepared as reference compounds (Scheme 1).



Scheme 1. Aliphatic and aromatic *N*-nitrosamines in this study

N-Nitroso compounds are known to take planar structures, because the rotational barriers of the N–NO bond are evaluated to be of similar magnitude to those of amides.[17,19,20] This can be understood in terms of the resonance structures (Scheme 2), which represent the partial double bond character of the N–N(O) bond, in a similar manner to the N–C(O) bond in amides. Rotational barriers in solution, the free energy of activation ($\Delta G_c^{\ddagger}$), of the *N*-nitrosamines can be evaluated from the slow exchange peak separation ($\Delta\nu$) and the coalescence temperature (T_c) of the protons adjacent to the amino group in the ^{1}H NMR spectra (Table 1).[15–17] The values ($\Delta G_c^{\ddagger}$) of the *N*-nitroso derivatives of 7-azabicyclo[2.2.1]heptanes (**1**, **2** and **3**) are apparently smaller than those of the monocyclic five-membered *N*-nitrosamines (**10** and **11**). This result suggests a reduction of the resonance contribution of the N–NO bond, depicted in Scheme 2, in the *N*-nitroso derivatives of the 7-azabicyclo[2.2.1]heptane motif. The rotational barriers of the isoindoline **8** and other monocyclic *N*-nitrosamines (**9** and **12**–**14**) are also estimated to be more than 20–21 kcal/mol. The rotational barrier of *N*-nitrosoazetidine **9** is comparable to those of five-membered ring systems (**10** and **11**), the value being consistent with the previous result (20.5 kcal/mol).[15] The dibenzo derivatives (**4**, **5**, **6** and **7**) also have small rotational barriers (Table 1), suggesting a reduction of the resonance in the N–NO bond (Scheme 2).[15]

N-Nitrosamines can produce NO through a homolytic cleavage of the N–NO bond, and also can form



Scheme 2. Resonance models of planar nitrogen of amides and *N*-nitrosamines

Table 1
Rotational barriers of *N*-nitrosamines

compound	solvent [a]	Tc (°C) [b]	$\Delta G_c^{\ddagger}$(kcal/mol) [c]
1	A	71.2	16.6
2	B	36.9	15.1
3	B	53.6	15.8
4	B	52.7	16.0
5	B	47.2	15.4
6	B	36.1	14.6
7	A	37.2	14.7 [d]
8	C	>170.4 [e]	>20.6
9	C	158.0	20.1
10	C	157.2	21.5
11	C	>170.1 [e]	>20.6
12	C	>170.1 [e]	>21.1
13	C	>170.0 [e]	>20.6
14	C	>170.1 [e]	>20.8

a) A: $CDCl_2CDCl_2$; B: $CDCl_3$; C: $C_6D_5NO_2$.
b) Errors: ± 1.0 °C. Temperatures were calibrated by means of a standard method.[24]
c) Rotational barriers ($\Delta G_c^{\ddagger}$) were obtained on the basis of the difference in chemical shifts of the two bridgehead proton signals and the coalescence temperature in proton NMR spectroscopy. Errors: ± 0.3 kcal/mol.
d) In solvent B; T_c : 37.9 °C; $\Delta G_c^{\ddagger}$: 14.6 kcal/mol.
e) The maximum measurable with the apparatus.

NO^+ through a heterolytic cleavage of the relevant bond.[14] Release of NO or NO^+ from *N*-nitrosamines in solution can be detected with the Griess method.[21] The visible absorption at 595 nm of the resultant red dye, formed upon a diazo coupling of the Griess reagents, allows evaluation of the amount of the relevant species (NO or NO^+) formed by cleavage of the N–NO bonds of the *N*-nitrosamines (Fig. 1). Although the monocyclic aliphatic *N*-nitrosamines with five (**8**, **10** and **11**), six (**12**), seven (**13**) and eight (**14**)-membered rings, and even the four-membered *N*-nitrosoazetidine (**9**), are practically negative in the Griess assay, the *N*-nitroso derivatives (**2**, **3**, **4**, **5**, **6** and **7**) of the 7-azabicyclo[2.2.1]heptane motif are positive (weakly positive in the case of **1**). Some of these bicyclic derivatives are superior to the aromatic *N*-nitrosamines (**15** and **16**) and authentic NO donors (NOC12, NOC18 and SNAP)[22]

(Fig. 1). The present results strongly suggest that the low rotational barriers of the N–NO bond of the aliphatic *N*-nitrosamines, which reflect decreased *N*-nitroso resonance, are related to the facile N–NO bond cleavage.[23]

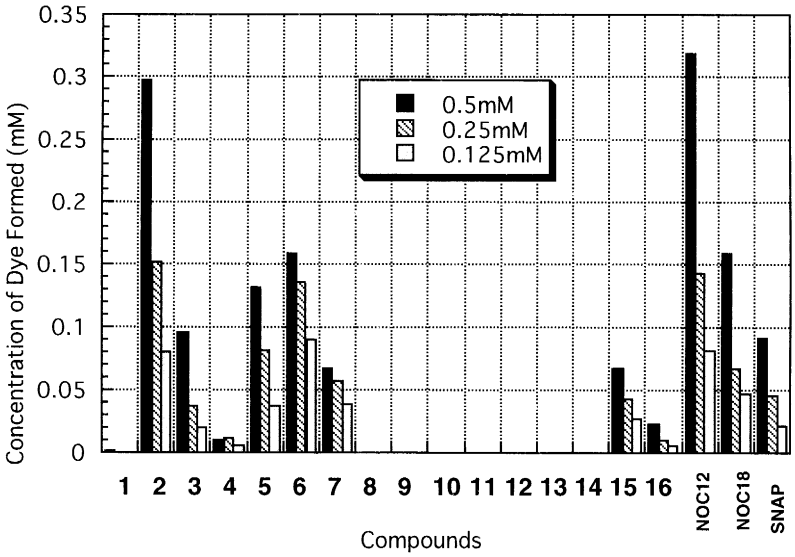


Fig. 1. Griess assay results, reflecting the ease of N–NO bond cleavage of the *N*-nitrosamines. After 5 h at 37°C[21]

In the Griess assay which reflects the ease of N–NO bond cleavage, the fused benzo group and the electron-withdrawing groups, such as an aromatic nitro group, the ester groups and the *N*-phenylimido group, seem to encourage N–NO bond cleavage of the *N*-nitroso derivatives of the 7-azabicyclo[2.2.1]heptanes (in Fig. 1: **2**, **3**>**1**; **5**, **6**, **7**>**4**).[21]

In summary, the N–NO bond of aliphatic *N*-nitroso derivatives of 7-azabicyclo[2.2.1]heptanes tends to be weak. The generality of these structural features of *N*-nitrosamines of 7-azabicyclo[2.2.1]heptanes, the relevant structural origins of the facile N–NO bond cleavage, and the detection of the species formed by N–NO bond cleavage are under investigation.[25]

Acknowledgements

We thank Dr. Hidetoshi Hayashi, Department of Hygienic Chemistry, Faculty of Pharmaceutical Sciences, Nagoya City University, for help with the Griess assay.

References

1. (a) Murad, F. *Angew. Chem., Int. Ed.* **1999**, *38* 1856–1868. (b) Ignarro, L. J. *Angew. Chem., Int. Ed.* **1999**, *38*, 1882–1892. (c) Furchgott, R. F. *Angew. Chem., Int. Ed.* **1999**, *38*, 1870–1880.
2. Pfeiffer, S.; Mayer, B.; Hemmen, B. *Angew. Chem., Int. Ed.* **1999**, *38*, 1714–1731.
3. (a) Stamler, J. S.; Jaraki, O.; Osborne, J.; Simon, D. I.; Keaney, J.; Vita, J.; Singel, D.; Valeri, C. R.; Loscalzo, J. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 7674–7677. (b) Stamler, J. S.; Singel, D. J.; Loscalzo, J. *Science* **1992**, *258*, 1898–1902.
4. Williams, D. L. H. *Acc. Chem. Res.* **1999**, *32*, 869–876.
5. (a) Toda, N.; Okamura, T. *J. Hypertension* **1996**, *14*, 423–434. (b) Saders, K. M.; Ward, S. M. *Am. J. Physiol*. **1992**, *262*, G379–G392.

6. *Advances in Phamacol.*; Ignarro, L.; Murad, F., Eds. Nitric oxide, biochemistry, molecular biology, and therapeutic implications. Academic Press: New York, 1995; Vol. 34, pp. 1–516.
7. Loeppky, R. N.; Michejda, C. J. *N-Nitrosamines and Related N-Nitroso Compounds*; American Chemical Society Symposium Series 553, American Chemical Society: Washington, DC, 1994.
8. Oh, S. M. N.Y. F.; Williams, D. L. H. *J. Chem. Soc., Perkin Trans. 2* **1989**, 755–758.
9. Castro, A.; Leis, J. R.; Pena, M. E. *J. Chem. Soc., Perkin Trans. 2* **1989**, 1861–1866.
10. Galtress, C. L.; Morrow, P. R.; Nag, S.; Smalley, T. L.; Tschantz, M. F.; Vaughn, J. S.; Wichems, D. N.; Ziglar, S. K.; Fishbein, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 1406–1411.
11. Santala, T.; Fishbein, J. C. *J. Am. Chem. Soc.* **1992**, *114*, 8852–8857.
12. Tanno, M.; Sueyoshi, S.; Miyata, N.; Nakagawa, S. *Chem. Pharm. Bull.* **1996**, *44*, 1849–1852. The authors reported a detection of NO formed by N–NO bond cleavage of aromatic *N*-nitrosoureas.
13. Tanno, M.; Sueyoshi, S.; Miyata, N.; Umehara, K. *Chem. Pharm. Bull.* **1997**, *45*, 595–598.
14. Cheng, J.-P.; Xian, M.; Wang, K.; Zhu, X.; Yin, Z.; Wang, P. G. *J. Am. Chem. Soc.* **1998**, *120*, 10266–10267.
15. Cooney, J. D.; Brownstein, S. K.; Apsimon, J. W. *Can. J. Chem.* **1974**, *52*, 3028–3036. High rotational barriers of the N–NO bond (23 kcal/mol<) of different bicyclic *N*-nitrosamines were also reported.
16. Harris, R. K.; Pryce-Jones, T.; Swinbourne, F. J. *J. Chem. Soc., Perkin Trans. 2* **1980**, 476–482.
17. (a) Mannschreck, A.; Münsch, H.; Mattheus, A. *Angew. Chem. In. Ed. Engl.* **1966**, *5*, 728. (b) Mannschreck, A.; Münsch, H. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 984–985. (c) Oki, M. *Applications of Dynamic NMR Spectroscopy to Organic Chemistry, Vol. 4*; VCH Publishers: Deerfield, USA, 1985.
18. *N*-Acyl and *N*-sulfonyl amides of 7-azabicyclo[2.2.1]heptanes exhibited nitrogen pyramidalization: (a) Ohwada, T.; Achiwa, T.; Okamoto, I.; Shudo, K.; Yamaguchi, K. *Tetrahedron Lett.* **1998**, *39*, 865–868. (b) Ohwada, T.; Okamoto, I.; Shudo, K.; Yamaguchi, K. *Tetrahedron Lett.* **1998**, *39*, 7877–7880.
19. Gropen, O.; Skancke, P. N. *Acta Chem. Scand.* **1971**, *25*, 1241–1249.
20. Gdaniec, M.; Milewska, M. J.; Polonski, T. *J. Org. Chem.* **1995**, *60*, 7411–7418.
21. The Griess reagents contain a 10% aqueous solution of sulfanilamide in distilled water and phosphoric acid, and a 1% aqueous solution of *N*-(1-naphthyl)ethylenediamine dihydrochloride in distilled water. To a mixture of an equal volume of the above two solutions, a solution of an *N*-nitrosamine in DMSO was added to obtain a final concentration of the *N*-nitrosamine of 0.50, 0.25 and 0.125 mM, in which 10% (v/v) of DMSO in water was contained. After 5 h at 37°C, the absorbance at 595 nm was measured. The absorption increased time-dependently. Compounds **4**, **5**, **6** and **7** did not dissolve completely in the Griess reagents in the cases of 0.5 and 0.25 mM. See: Green, L. C.; Wagner, D. A.; Glogowski, J.; Skipper, P. L.; Wishnok, J. S.; Tannenbaum, S. T. *Anal. Chem.* **1982**, *126*, 131–138.
22. NOC12 (*N*-ethyl-2-(1-ethyl-2-hydroxy-2-nitrosohydrazino)ethanamine); NOC18 (2,2′-(hydroxynitrosohydrazino)bis-ethanamine), see: Hrabie, J. A.; Klose, J. R.; Wink, D. A.; Keefer, L. K. *J. Org. Chem.* **1993**, *58*, 1472–1476. SNAP (*S*-nitroso-*N*-acetyl-DL-penicillamine), see: Field, L.; Dilts, R. V.; Ravichandran, R.; Lenhert, P. G.; Carnahan, G. E. *J. Chem. Soc., Chem. Commun.* **1978**, 249–250.
23. *N*-Nitrosoindoline (**15**) and *N*-methyl-*N*-phenylnitrosamine (**16**) exist as a single conformational isomer. See: Looney, C. E.; Phillips, W. D.; Reilly, E. L. *J. Am. Chem. Soc.* **1957**, *79*, 6136–6142. The rotational barrier of an aromatic *N*-nitrosamine, *N*-nitrosodiphenylamine, was reported to be 19.1 kcal/mol by: Forlani, L.; Lunazzi, L.; Macciantelli, D.; Minguzzi, B. *Tetrahedron Lett.* **1979**, 1451–1452.
24. Ammann, C.; Meier, P.; Merbach, A. E. *J. Magn. Reson*, **1982**, *46*, 319–321.
25. A preliminary study of the Griess assay in different pH media suggests that acidic conditions are required to enhance N–NO bond cleavage of the relevant *N*-nitrosamines.