Tetrahedron Letters No. 19, pp 1613 - 1616, 1977. Pergamon Press. Printed in Great Britain.

TRANSNITROSATION BY ALIPHATIC NITROSAMINES

*Sandra S. Singer, William Lijinsky, and George M. Singer
NCI FREDERICK CANCER RESEARCH CENTER, Frederick, Md., U.S.A., 21701

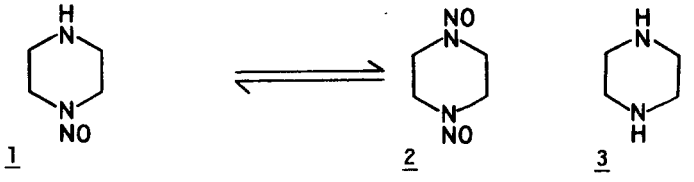
(Received in USA 15 February 1977; received in UK for publication 28 March 1977

The carcinogenic activity of nitrosamines is well documented[1], yet little is known about the mechanism of action or even the chemistry of these compounds. Some investigations of the aliphatic nitrosamines, e.g. dimethylnitrosamine, suggest they could act as alkylating agents[2], possibly *via* carbonium ions. However, other pathways are conceivable.

The common feature of all nitrosamines is the nitroso group itself and the special chemical properties it imparts to the molecule. Transnitrosation, the transfer of a nitroso group from a nitrosamine to an amine (or other receptor) has been demonstrated in a few specific cases[3], but there is no evidence in the literature that any aliphatic nitrosamine could effect a facile transnitrosation[3,4].

Rats fed high doses of the mononitrosopiperazine (1) showed the same tumor spectrum as rats fed much lower doses of dinitrosopiperazine (2)[5]. Moreover, an old sample (*ca.* 8 years) of mononitrosopiperazine contained a white precipitate which was subsequently shown by mass spectroscopy and high pressure liquid chromatography (HPLC) to be dinitrosopiperazine. This suggested that a transnitrosation could be occurring, either as a direct uncatalyzed reaction, or in the biological test, perhaps catalyzed by the stomach acid of the rats fed mononitrosopiperazine. (eq. 1)

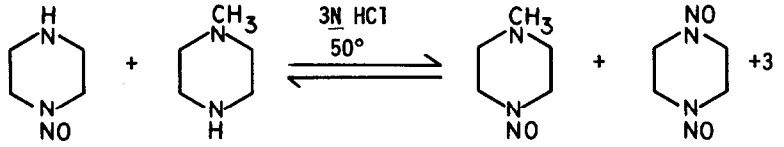
We wish to report that we have discovered mild conditions under which a number of alicyclic nitrosamines undergo facile transnitrosation. In 0.1N HCl (at 50°) several piperazines, morpholine, and proline, and their nitroso derivatives have been found to be suitable donors and recipients.



1 2 3 (Eq. 1)

Initial experiments with mononitrosopiperazine (1) showed that in 3N HCl at 50° disproportionation was occurring, reaching an equilibrium ratio of mono- to dinitrosopiperazine of 2.4 within 60-75 minutes.

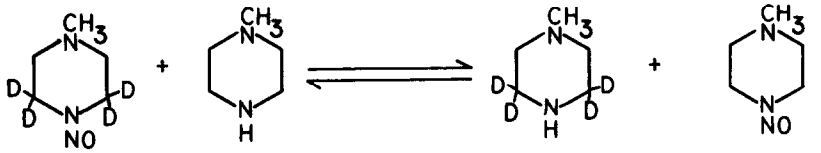
We then investigated the possibility whether this nitrosotransfer might occur with other secondary amines. Addition of N-methylpiperazine (4) to a solution of mononitrosopiperazine in 3N HCl gave both 4-methyl-1-nitrosopiperazine (5) and 2 as products (eq. 2)



1 4 5 2 (Eq. 2)

4-Methyl-1-nitrosopiperazine is formed first, and dinitrosopiperazine is formed more slowly, suggesting that the 4-methylpiperazine competes more effectively for the transferring nitroso species.

4-Methyl-1-nitrosopiperazine itself is also a good nitroso-transfer reagent. 2,2,6,6-Tetradeutero-4-methyl-1-nitrosopiperazine (synthesized by the method of Keefer and Fodor[6]) and N-methylpiperazine in 3N HCl at 50° for 45 minutes gave an equilibrium mixture with a mass spectrum (via glc-ms) showing molecular ions at m/e 133 and m/e 129 corresponding to the tetradeutero-(6) and undeuterated (5) compounds, respectively, in a 1:1 ratio.



6 4 7 5 (Eq. 3)

When 1 was heated in 3N HCl at 50° with morpholine, a small amount of nitrosomorpholine was formed. The reaction was quite slow, and 2 was the main product. When 1 was allowed to react with piperidine under these conditions, no nitrosopiperidine was found.

Pseudo first-order rates of denitrosation were determined for mono- and dinitroso-piperazine, 4-methyl-1-nitrosopiperazine, nitrosomorpholine, and nitrosohexamethyleneimine. (Table 1). In 3N HCl, at 50°, with equimolar ammonium sulphamate present as a trapping agent, the order of reactivity was as follows: 4-methyl-1-nitrosopiperazine>mononitrosopiperazine>> dinitrosopiperazine. Nitrosomorpholine and nitrosohexamethyleneimine did not decompose under these conditions.

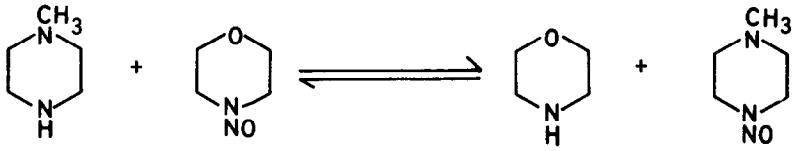
Table 1
Pseudo first order rates for denitrosation of nitrosamines with $NH_4SO_3NH_2$

Compound	Acid	pH	k_o
5	3N HCl	-0.5	8.2×10^{-4} sec.$^{-1}$
1	3N HCl	-0.5	3.5×10^{-4} sec.$^{-1}$
2	3N HCl	-0.5	1.3×10^{-4} sec.$^{-1}$

We next considered the question of catalysis by nucleophiles such as Cl^-, BR^-, and SCN^-.

At pH 1.4, where the transnitrosation occurs only very slowly (mononitrosopiperazine gave only a trace of 2 after 24 hours), addition of 1M Cl^- accelerated the reaction only slightly. With added bromide (1M), the reaction reached equilibrium in 6 hours (k_o = 2.5 x 10^{-5} $sec.^{-1}$). However, when thiocyanate (1M) was the added nucleophile, the reaction reached equilibrium in 15 minutes (k_o = 5.7 x 10^{-4} $sec.^{-1}$). At pH 2 the reaction proceeds more slowly, the initial rate for disappearance of mononitrosopiperazine being 1.7 x 10^{-4} $sec.^{-1}$. At pH 3, only a trace of 2 was found after 24 hours (with 1M SCN^-). The reaction is also dependent on thiocyanate concentration: when 0.1M thiocyanate is the added salt, the initial rate for disappearance of 1 is 1.3 x 10^{-4} $sec.^{-1}$ at pH 1.4.

Challis demonstrated that the decomposition of nitrosomorpholine is catalyzed in polar medium by thiocyanate[3,4]. We have found that nitrosomorpholine can also act as a transnitrosating agent in aqueous 1M SCN^- at pH 1.5. Equimolar N-methylpiperazine was used as the nitroso-recipient. After two hours the reaction mixture contained a 2:1 mixture of nitrosomorpholine and 4-methyl-1-nitrosopiperazine (eq. 4).



4 8 5 9 (Eq. 4)

Table 2
Initial rates for disappearance of mononitrosopiperazine (0.05M)

Acid	pH[a]	Catalyst	k_o ($sec.^{-1}$)
0.1N HCl	1.4	SCN^- (1M)	5.7 x 10^{-4}
0.1N $HClO_4$	1.4	SCN^- (1M)	4.3 x 10^{-4}
0.1N HCl	1.8	SCN^- (1M)	1.6 x 10^{-4}
0.1N HCl	2.0	SCN^- (1M)	1.7 x 10^{-4}
0.1N HCl	1.4	SCN^- (0.1M)	1.3 x 10^{-4}
0.1N HCl	1.4	Br^- (1M)	2.5 x 10^{-5}
3N HCl	-0.5	---------	1.4 x 10^{-4}
3N $HClO_4$	-0.5	Cl^- (3M)	8.9 x 10^{-4}

a - pH adjusted by addition of NaOH

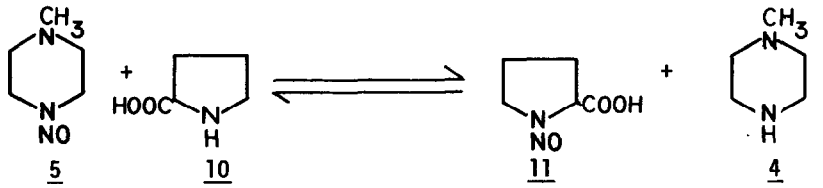
After 24 hours, this reaction mixture contained less nitrosomorpholine than 4-methyl-1-nitrosopiperazine (a ratio of 1:2), and the molar total of nitrosamines was 87% of theoretical. In 24 hour or longer reactions, some sulfur precipitates, indicating that thiocyanate is decomposing.

Hydrochloric acid has been used in these reactions in order to simulate conditions found in the stomach. To determine whether HONO or NOX was the free nitrosating species, the mononitrosopiperazine disproportionation reaction was carried out in perchloric acid (since $NOClO_4$ does not exist as a molecular species[7]). In 3N $HClO_4$ at 50°, no dinitrosopiperazine was formed, although the Griess reagent color test qualitatively indicated the presence of a small amount of free nitrite. After 4 hours at 50°, 3M Cl^- was added (as NaCl). The disproportionation proceeded extremely rapidly (k_o = 8.9 x 10^{-4} $sec.^{-1}$), reaching equilibrium

rapidly. In 3N HCl at 50°, the reaction reached equilibrium in 60 minutes, k_o = 1.4 x 10^{-4} sec.$^{-1}$. The greater velocity of the reaction in perchloric acid with added sodium chloride is probably due to the greater ionic strength of the reaction medium in that case. In a pH 1.4 $HClO_4$ solution, there was also no reaction at 50° after 4 hours. Addition of 1M SCN^- induced a rapid reaction (k_o = 4.3 x 10^{-4} sec.$^{-1}$).

The lack of reaction in perchloric acid clearly shows that neither free HONO nor N_2O_3 is the active species in these transnitrosation reactions. Direct S_N2 displacement by the recipient amine on the donor nitrosamine is also ruled out by the lack of reaction in pH 1.4 $HClO_4$. More extensive kinetic studies will establish whether nitrosotransfer proceeds through acid catalyzed denitrosation and subsequent formation of NOX which then nitrosates the recipient amine, or whether the nucleophile participates in an S_N2 displacement to give NOX.

Preliminary results indicate that the nitrosotransfer reaction may be more general than we originally anticipated. Proline will accept a nitroso group from 4-methyl-1-nitroso-piperazine at pH 1.4 in the presence of 1M SCN^-. Furthermore nitrosoproline will act as a donor under the same conditions. (eq. 5)



5 10 11 4 (Eq. 5)

In both cases, the same equilibrium mixture is reached eventually (nitrosoproline/4-methyl-1-nitrosopiperazine = 0.9)

The relative rates of denitrosation of all compounds now known capable of nitroso group transfer are in the approximate order: nitrosodiphenylamine~nitrosocarbazole>1-nitroso-4-methylpiperazine>mononitrosopiperazine>nitrosoproline>dinitrosopiperazine>nitrosomorpholine. Of possibly great importance, if the non-carcinogenic compounds on the list were present in the stomach under the right conditions (suitable pH, or presence of thiocyanate, which is found in saliva, and the presence of a nitroso-recipient), these non-carcinogens could give rise to potent carcinogens by transnitrosation.

Research sponsored by the National Cancer Institute under Contract No. NO1-CO-25423 with Litton Bionetics, Inc.

REFERENCES

1. Magee, P.N. and Barnes, J.M., Advanc. Cancer Res., 10, 163 (1967).
2. Magee, P.N. and Lee, K.Y., Biochem. J., 91, 35 (1964).
3. Buglass, A.J., Challis, B.C., and Osborne, M.R., "N-Nitroso Compounds in the Environment", p. 94, IARC monograph #9, P. Bogovski and E.A. Walker, ed., Lyon (1974).
4. Challis, B.C., and Osborn, M.R., Chem. Comm., 518, (1972).
5. Love, L., Lijinsky, W., Keefer, L., and Garcia, H., Z. Krebsforsch. In press.
6. Keefer, L.K., and Fodor, C.H., J. Am. Chem Soc., 92, 5747 (1970).
7. Ridd, J.H., Q. Rev., 15, 418 (1961).