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Production of nitroxyl (HNO) at biologically relevant temperatures from the retro-Diels–Alder reaction of *N*-hydroxyurea-derived acyl nitroso-9,10-dimethylanthracene cycloadducts

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Abstract

Retro Diels–Alder reaction of *N*-hydroxyurea-derived acyl nitroso compound-9,10-dimethylanthracene cycloadducts produce acyl nitroso compounds that react with nucleophiles to form nitrous oxide, which indicates the intermediacy of nitroxyl. These results identify these molecules as a new group of nitroxyl delivery agents.

Keywords: nitric oxide; nitroxyl; acyl nitroso compounds; *N*-hydroxyureas; retro-Diels–Alder.

Accumulating evidence indicates the biological importance of nitroxyl (HNO/NO^-), the one-electron reduced form of nitric oxide (NO). Nitroxyl releasing compounds relax pre-constricted rabbit aortic rings in vitro mimicking the action of NO donors.[1] Nitroxyl causes double stranded DNA cleavage resulting in tumor cell cytoxicity.[2] Nitroxyl inhibits aldehyde dehydrogenase and represents the active agent in the mechanism of action of the clinically used alcohol deterrent cyanamide.[3] Nitroxyl has also been implicated in biosynthetic NO production as the possible initial product from the nitric oxide synthase (NOS) catalyzed oxidation of L-arginine.[4] The intrinsic biological activity of nitroxyl may partially explain how nitric oxide elicits responses in so many widely different physiological systems.[5]

With the emerging picture that nitroxyl plays a significant biological role, compounds which donate nitroxyl will be of increasing interest and importance as both pharmacological tools and therapeutic agents. The hydrolysis of acyl nitroso species, such as **1** (Scheme 1), represents a reliable chemical strategy for nitroxyl production.[6] Identification of nitrous oxide (N_2O), which

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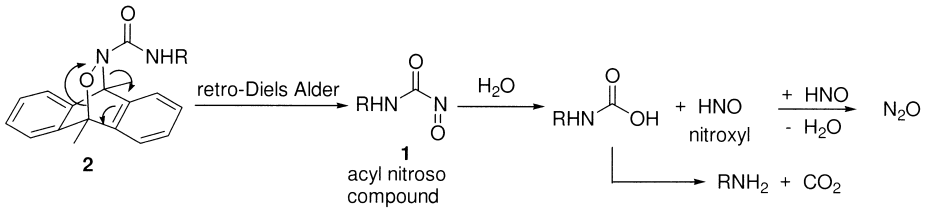
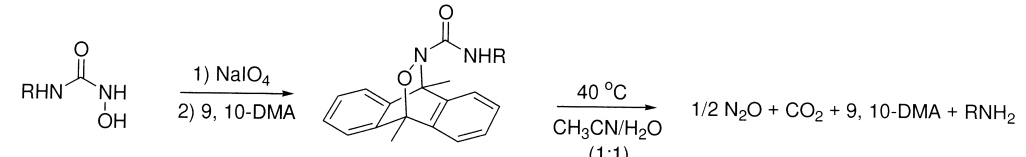
forms through nitroxyl dimerization followed by dehydration, provides evidence for nitroxyl formation (Scheme 1).[7] Retro-Diels–Alder decomposition of properly constructed cycloadducts provides a clean method for the generation of acyl nitroso species.[8] Nitroxyl production from such reactions occurs at elevated temperatures or with catalytic antibody assistance.[9] We wish to report that cycloadducts of acyl nitroso compounds derived from *N*-hydroxyureas (**2**, R = NHR) and 9,10-dimethylanthracene (9,10-DMA) thermally decompose with nitroxyl formation at biologically relevant temperatures without the requirement of catalysis (Scheme 1).



Scheme 1.

Periodate oxidation of the *N*-hydroxyureas (**3a–d**) in the presence of 9,10-DMA produced the *N*-hydroxyurea-derived DMA cycloadducts (**4a–d**, Scheme 2) in good yield.[10,11] Thermal decomposition at 40°C of these cycloadducts in a mixture of acetonitrile and water (1:1) generated N_2O, carbon dioxide (CO_2), 9,10-DMA and the corresponding amine (Scheme 2).[12] Enzymatic identification of ammonia from **4a** was not successful in the organic solvent mixture. Nitrous oxide formation provides strong evidence for nitroxyl intermediacy and Table 1 summarizes the yields of N_2O and CO_2 produced. The non-substituted and *n*-butyl-substituted cycloadducts (**4a** and **4c**) produced the greatest amount of N_2O while those containing aromatic rings (**4b** and **4d**) produced a significantly smaller amount (Table 1). Thermal decomposition of **4a** at 40°C in a mixture of acetonitrile and methanol (1:1) generated N_2O (30%) and 9,10-DMA but did not produce CO_2 (Table 1, Entry 2). Only small amounts (< 5%) of nitrite (NO_2^-), the oxidative decomposition product of NO,[13] were detected in these reactions (Table 1).



3a, R = H
3b, R = -CH_2Ph
3c, R = -$CH_2CH_2CH_2CH_3$
3d, R = -Ph

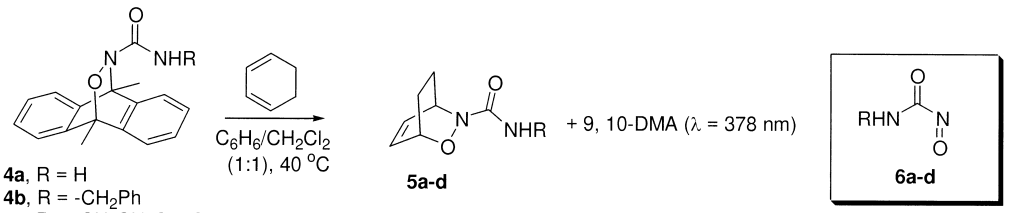
4a, R = H, 41%
4b, R = -CH_2Ph, 93%
4c, R = -$CH_2CH_2CH_2CH_3$, 70%
4d, R = -Ph, 73%

Scheme 2.

Thermal decomposition of cycloadducts **4a–d** at 40°C in a mixture of methylene chloride (CH_2Cl_2) and benzene in the presence of 1,3-cyclohexadiene provided further information regarding the mechanism of these reactions. The reaction of **4a–d** under these conditions produced 9,10-DMA and the 1,3-cyclohexadiene cycloadducts **5a–d**, which are thermally stable at 40°C (Scheme 3). The isolation and characterization of **5a–d** provides strong evidence for acyl nitroso intermediates (**6a–d**) during the decomposition of **4a–d** (Scheme 3).[14]

Table 1
Product yields from the thermal decomposition of cycloadducts **4a**–**4d** at 40°C

			% Yield				
Entry	Compound	Solvent	N_2O	CO_2	NO_2^-	9,10-DMA	RNH_2
1	**4a**	CH_3CN/H_2O	44	44	5	+	-
2	**4a**	CH_3CN/CH_3OH	30	0	1	+	-
3	**4b**	CH_3CN/H_2O	15	17	1	+	+
4	**4c**	CH_3CN/H_2O	56	45	1	+	+
5	**4d**	CH_3CN/H_2O	14	45	1	+	+



4a, R = H
4b, R = -CH_2Ph
4c, R = -$CH_2CH_2CH_2CH_3$
4d, R = -Ph

Scheme 3.

Spectrophotometric measurement of 9,10-DMA formation ($\lambda = 378$ nm) during these reactions indicate that cycloadducts (**4a**–**d**) decompose in a first order manner (Scheme 3). The rate of the decomposition did not change as the concentration of 1,3-cyclohexadiene increased (Table 2, Entry 2). These results indicate that the initial dissociation of **4a**–**d** represents the rate limiting step and Table 2 summarizes the first order rate constants and half-lives for these reactions. The alkyl-substituted cycloadducts **4b**–**c** decomposed 2–4 times faster than the non-substituted cycloadduct (**4a**, Table 2, Entries 1, 3–4). The aromatic-substituted cycloadduct **4d** decomposed 10 times faster than **4a** (Table 2, Entry 5). Addition of electron withdrawing and donating groups to the phenyl ring of **4d** did not significantly change the rate of decomposition indicating that the increase in decomposition rate of **4d** compared to **4a**–**c** is most probably related to steric rather than electronic factors (Table 2, Entries 6–7).

Table 2
First order rate constants and $t_{1/2}$ for the thermal decomposition of **4a**–**d** at 40°C

Entry	Cycloadduct	Equiv. of 1, 3-cyclohexadiene	K (s^{-1})	$t_{1/2}$ (hr)
1	**4a**	1	7.5×10^{-5}	2.6
2	**4a**	10	7.2×10^{-5}	2.7
3	**4b**	2	1.4×10^{-4}	1.4
4	**4c**	2	2.7×10^{-4}	0.72
5	**4d**	2	7.5×10^{-4}	0.26
6	**4d**-*para*-NO_2	2	5.5×10^{-4}	0.34
7	**4d**-*para*-OCH_3	2	7.1×10^{-4}	0.27

These results demonstrate that cycloadducts of acyl nitroso compounds derived from *N*-hydroxyureas and 9,10-DMA (**4a**–**d**) undergo retro-Diels–Alder reactions to produce acyl nitroso species (**6a**–**d**) at biologically relevant temperature with reaction half-lives between 0.25 and 2.7 h

(Scheme 3, Table 2). Hydrolysis of **6a**–**d** produces nitroxyl and carbamic acids that decompose to carbon dioxide and the corresponding amines (Schemes 1 and 2). Nitrous oxide formation during these reactions provides strong evidence for nitroxyl intermediacy.[7] Methanolysis of **6a**, to presumably form a methyl carbamate, would explain the lack of CO_2 formation during the decomposition of **4a** in a mixture of acetonitrile and methanol (Table 1, Entry 2). As the nature of the *N*-hydroxyurea-substituent influences the rate of these reactions, the possibility of designing and preparing nitroxyl delivery agents with varied release profiles exists. In summary, cycloadducts **4a**–**d** cleanly liberate nitroxyl at biologically relevant temperatures and neutral pH in the absence of additional reagents or enzymatic activation or catalysis. Such compounds represent new nitroxyl delivery agents that may be useful as tools for differentiating the actions of nitroxyl and nitric oxide in biological systems.

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14. Compounds **4a**–**d** and **5a**–**d** gave satisfactory ^{1}H and ^{13}C NMR spectra and elemental analyses. For example: compound **4a**: mp 181–182.5, lit. 182–183;[11] ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.57–7.18 (m, 8H) 2.66 (s, 3H), 2.25 (s, 3H); ^{13}C NMR (50 MHz $CDCl_3$) δ 163.7, 141.4, 140.7, 127.9, 127.5, 122.3, 120.8, 79.7, 64.5, 17.5, 15.8. Compound **5a**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 6.52 (m, 2H) 5.22 (br s, 2H), 4.90 (m, 1H), 4.67 (m, 1H), 2.13 (m, 4H); anal. calcd for $C_7H_{10}N_2O_2$: C, 54.54; H, 6.54; N, 18.17. Found: C, 54.71; H, 6.94; N, 18.43. Cycloadducts **4a**–**d** slowly decompose to 9,10-DMA at room temperature. Storage of these compounds in a freezer prevents thermal decomposition.