

## Direct Observation of an Acyl Nitroso Species in Solution by Time-Resolved IR Spectroscopy

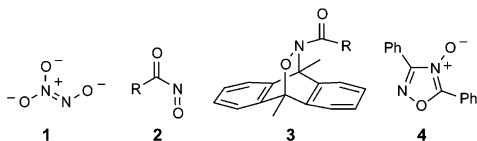
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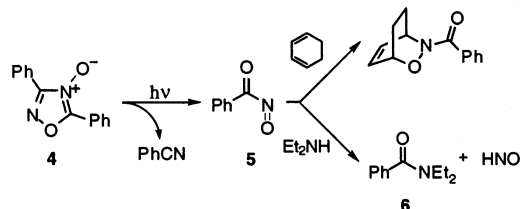
The recent and resurgent interest in the biological chemistry of nitrogen oxides has focused mainly on nitric oxide (NO).<sup>1</sup> Much of the fundamental chemistry of this natural free radical has been worked out long ago. On the other hand, much less is known about the nitroxyl system (NO<sup>-</sup>/HNO), the one-electron reduced congener of NO.<sup>2,3</sup> For instance, the uncertainty concerning the pK<sub>a</sub> of HNO<sup>4,5</sup> has only recently been clarified. A value of approximately 11 has been obtained by several methods.<sup>6,7</sup> Direct observation of HNO has been limited mainly to gas-phase<sup>8,9</sup> and matrix<sup>10</sup> studies. Currently, studies use prodrugs of HNO (that decompose spontaneously under physiological conditions) to monitor its pharmacological effects.<sup>11–13</sup> Further knowledge of the fundamental solution-phase chemistry of HNO will certainly be relevant to the understanding of its physiological efficacy.

The most common chemical precursor to the nitroxyl system is Angeli's anion (**1**), which decomposes to NO<sup>-</sup> and NO<sub>2</sub><sup>-</sup> both thermally<sup>14</sup> and photochemically.<sup>15</sup> Other precursors include *N*-hydroxybenzenesulfonamide (Piloty's acid)<sup>16</sup> and the naturally occurring *S*-nitrosothiols.<sup>17</sup> Recent attention has been given to acyl nitroso compounds (**2**), transient electrophiles that react with nucleophiles to yield HNO.<sup>18</sup> Acyl nitroso compounds are most commonly generated by either the periodate oxidation of hydroxamic acids or the thermal fragmentation of Diels–Alder adducts.<sup>19–21</sup> King and co-workers have used dimethylantracene-based Diels–Alder adducts (**3**, R = NR<sub>2</sub>) to produce urea derivatives of **2** (R = NR<sub>2</sub>) at biologically relevant temperatures.<sup>22</sup> Nevertheless, the only spectroscopic evidence for **2** has been provided in the gas phase by Schwarz and co-workers using both charge reversal and neutralization–reionization mass spectrometry.<sup>23,24</sup> Herein, we report the first direct detection of an acyl nitroso compound (**2**, R = Ph) in solution by time-resolved infrared (TRIR) spectroscopy. We have monitored its reactions with diethylamine (DEA) and with 1,3-cyclohexadiene (CHD). We also report the direct detection of HNO, formed upon reaction with DEA.



Caramella and co-workers initially reported the photochemical fragmentation of 3,5-diphenyl-1,2,4-oxadiazole-4-oxide (**4**) to form benzoyl nitroside (**5**) and benzonitrile (Scheme 1).<sup>25</sup> Laser photolysis (355 nm, 5 ns, 4 mJ) of a solution of **4** in acetonitrile-*d*<sub>3</sub> produces the TRIR difference spectra shown in Figure 1a.<sup>26</sup> Analogous spectra are observed in dichloromethane. The transients are formed faster than the time-resolution of our instrument ( $k_{\text{obs}} > 2.0 \times 10^7 \text{ s}^{-1}$ ) and are stable for at least 180  $\mu\text{s}$  (Supporting Information).

Scheme 1



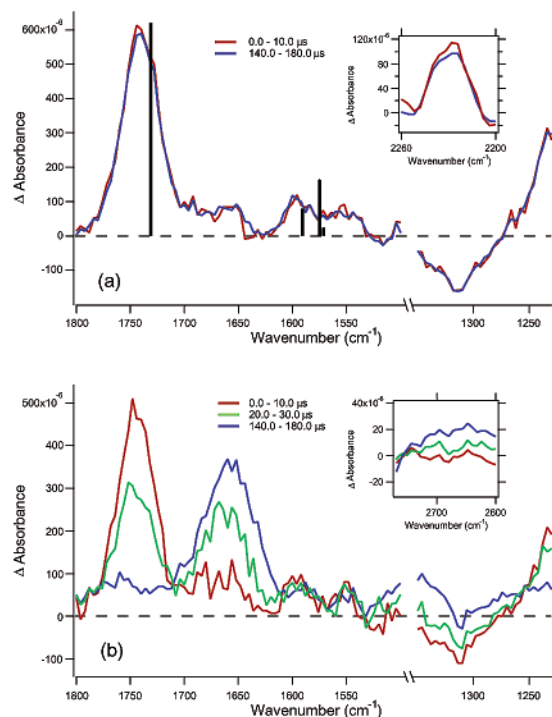
In addition to the bleaching of **4** at 1310 cm<sup>-1</sup>, we observe bands due to both benzonitrile (2265 cm<sup>-1</sup>) and acyl nitroso **5** (1735, 1590, 1560, and 1235 cm<sup>-1</sup>). To corroborate this latter assignment, we examined the B3LYP/6-31G\* calculated frequencies of **5** (scaled by 0.96<sup>27</sup>). These frequencies are in good agreement with the observed IR spectra, as shown in Figure 1a. Laser photolysis (266 nm, 5 ns, 2 mJ) of Diels–Alder adduct **3** (R = Ph), a known thermal precursor to **5**,<sup>19</sup> yields a similar IR band at 1735 cm<sup>-1</sup>, although the photochemistry here appears to be substantially more complicated and will require a more detailed study.

Consistent with literature reports,<sup>25</sup> we observe trapping of acyl nitroso **5** when generated in the presence of both added DEA and CHD in either acetonitrile-*d*<sub>3</sub> or dichloromethane (Scheme 1). The observed decay rates of **5** ( $k_{\text{obs}}$ ) are obtained by monitoring the signal at 1735 cm<sup>-1</sup> following excitation. Using the pseudo-first-order equation ( $k_{\text{obs}} = k_0 + k_{\text{q}}[\text{Q}]$ ), the second-order rate constants  $k_{\text{DEA}} = (1.3 \pm 0.5) \times 10^5 \text{ M}^{-1} \text{ s}^{-1}$  and  $k_{\text{CHD}} = (6.0 \pm 0.5) \times 10^3 \text{ M}^{-1} \text{ s}^{-1}$  are derived (Supporting Information). We estimate that the lifetime of **5** at infinite dilution ( $1/k_0$ ) in organic solution is on the order of 1 ms. At the high concentrations (> 10 M) required to observe trapping by water-*d*<sub>2</sub>, strong IR absorbance preclude accurate monitoring of the 1735 cm<sup>-1</sup> signal.

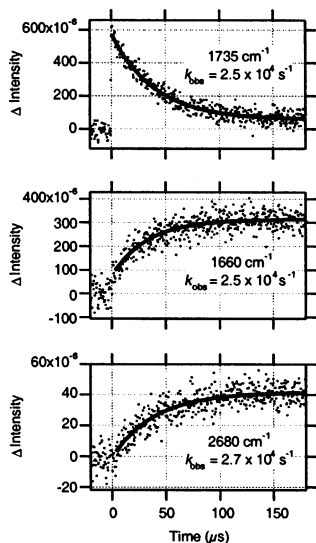
Laser photolysis of a solution of **4** in acetonitrile-*d*<sub>3</sub> with 0.15 M diethylamine produces the TRIR difference spectra shown in Figure 1b. Analogous spectra are observed in dichloromethane. Decay of the bands due to acylnitroso **5** are accompanied by the growth of a new band at 1660 cm<sup>-1</sup> with identical kinetics (Figures 1b, 2). The stable 1660 cm<sup>-1</sup> signal is assigned to *N,N*-diethylbenzamide (**6**) on the basis of comparison with authentic material. Additionally, we observe a weak broad signal around 2650 cm<sup>-1</sup> that also grows with identical kinetics (Figures 1b, 2). We tentatively assign this signal to HNO on the basis of the following observations. No signal is observed in the absence of added nucleophiles. The band is broadened and red-shifted compared to the N–H vibrational frequency of matrix-isolated (2717 cm<sup>-1</sup>)<sup>10</sup> and gas-phase (2684 cm<sup>-1</sup>)<sup>8</sup> HNO, as expected in solution with a high concentration of H-bond acceptors. We were unable to observe cleanly the N=O stretch expected near 1550 cm<sup>-1</sup> for HNO due to overlap with the N=O stretch of **5** as well as with aromatic vibrational modes of both **5** and **6**.

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**Figure 1.** TRIR difference spectra averaged over the time frames indicated following laser photolysis (355 nm, 5 ns, 4 mJ) of a solution of **4** (2.5 mM) in acetonitrile- $d_3$  (a) without and (b) with 0.15 M diethylamine. Negative signals are due to bleaching of the ground state, and positive signals are due to new transients or products. Insets show (a) the formation of benzonitrile and (b) the formation of HNO. The bars in (a) indicate calculated frequencies (B3LYP/6-31G\*, scaled by 0.96<sup>27</sup>). An intense C–C(O) stretch is also calculated at 1198 cm<sup>-1</sup> (not shown).



**Figure 2.** Kinetic traces observed at 1735, 1660, and 2680 cm<sup>-1</sup> following laser photolysis (355 nm, 5 ns, 4 mJ) of a solution of **4** (2.5 mM) and diethylamine (0.2 M) in acetonitrile- $d_3$ . The dotted curves are experimental data; the solid curves are the calculated best fits to a single-exponential function.

In summary, we provide the first direct detection and kinetic study of an acyl nitroso compound. Additionally, we present

preliminary evidence indicating direct IR detection of HNO. Water-soluble analogues of **4**, currently under development in our laboratory, could prove useful for the study of both the fundamental aqueous chemistry of nitroxyl as well as its potential applications in biology and medicine.

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**Supporting Information Available:** Details of the synthesis of precursors **3** (R = Ph) and **4**, kinetic traces for the formation of **5** in the absence of quencher, details of the derivation of second-order rate constants, and B3LYP/6-31G\* optimized geometry and frequencies of **5** (PDF). This material is available free of charge via the Internet at <http://pubs.acs.org>.

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