Nitroprusside, $\text{[Fe(CN)$_5$(NO)$_2$]}^{3-}$ (NP), was first synthesized in the middle of the 19th century, and it has been used as a qualitative indicator of sulfhydryl groups since its discovery.$^1$ Surprisingly, there exists no evidence for the structure of the transient “red products” (RPs) that are formed when NP reacts with thiolates, although they are widely postulated to be adducts of organothionitrites (RSNO). The hypotensive action of NP was first demonstrated 70 years ago,$^2$ and NP came into use as a drug more than 40 years ago.$^3$ The action of NO upon smooth muscle takes place intercellularly by binding to the active site of guanylate cyclase ($\text{GC}$).$^4$ It has been suggested that endogenous thiols are obligatory (as reductants or $\text{vis-à-vis}$ RSNO adducts) for the initiation of the hypotensive actions of NP.$^5$ We describe in vitro time-resolved IR spectra that for the first time evidence the molecular structures of species formed by one-electron reduction of NP and the RP in aqueous media.

NP can be reduced by one electron to $\text{[Fe(CN)$_5$(NO)$_3$]}^{3-}$ (NP$_1$$^-$). Earlier pulse radiolysis studies of the aqueous reduction of NP suggest NP$_1$$^-$ rapidly loses CN$^-$ to give $\text{[Fe(CN)$_5$(NO)$_2$]}^{3-}$ (NP$_2$$^-$).$^6$ Both NP$_1$$^-$ and NP$_2$$^-$ have been previously isolated by Na reduction of NP in anhydrous NH$_3$ and their IR spectra have been measured as Nujol mulls.$^7$ The crystal structure of (NBu$_4$)$_2$NP$_2$$^-$ has been determined.$^8$ We have studied the outer-sphere reduction of NP by several reagents including dithionite$^9$ and have for the first time measured the IR spectra of NP$_1$$^-$ and NP$_2$$^-$ in aqueous media. Equilibrium mixtures$^{10}$ of NP$_1$$^-$ and NP$_2$$^-$ can be prepared as a function of pH.$^7$ Since eq 2 involves the protonation of a strong base ($K = 2.0 \times 10^7$), the ratio of NP$_1$$^-$:NP$_2$$^-$ (eq 1, $K_1 = 6.6 \times 10^{-5}$) is determined by the [CN$^-$].$^13$ Thus, NP$_1$$^-$ and NP$_2$$^-$ are both observed for [NP]$_{\text{total}} = 1 - 10$ mM at neutral pH in the absence of added CN$^-$. The observed $\nu_{\text{CN}}$ of 1648 and 1800 cm$^{-1}$ for NP$_2$$^-$ and NP$_2$$^-$ (Figure 1)$^{14}$ is expected for a bent six-coordinate [MNO]$^7$ and linear five-coordinate [MNO]$^7$, respectively.$^{15}$

$$\text{[Fe(CN)$_5$(NO)$_3$]}^{3-} \rightleftharpoons \text{[Fe(CN)$_5$(NO)$_2$]}^{3-} + \text{CN}^- \quad (1)$$

$$\text{CN}^- \text{H}^+ \rightleftharpoons \text{HCN} \quad (2)$$

NP reacts rapidly with thiolate (not thiol)$^{16}$ to give an intense red solution. In the 150 years since this reaction was first discovered, there have been many suggestions regarding the structure of the chromophore,$^{17}$ but it is generally assumed that the RP is $\text{[Fe(CN)$_5$(RSNO)]}^{3-}$ (NP$_3$$^-$), the result of nucleophilic attack of RS$^-$ on the NO-based LUMO of NP.$^8$ We have measured the UV$^-$$^-$ visible and IR spectra that evolve after NP is reacted with EtSH$^-$. These studies have identified a band at 1380 cm$^{-1}$ (Figure 2, bottom) that correlates with loss of the red color (520 nm).$^{19,20}$ The band shifts to 1350 cm$^{-1}$ when $^{15}$NO-labeled NP ($^{15}$NP) is employed (Supporting Information).$^{21}$ Free RSNO compounds exhibit $\nu_{\text{NO}}$ stretches about 1500 cm$^{-1}$. Assuming RSNO compounds are $\pi$-acid ligands, the $\nu_{\text{NO}}$ stretch of RSNO might be expected to decrease upon binding to an electron-rich transition-metal moiety such as $\text{[Fe(CN)$_5$(NO)$_3$]}^{3-}$. In addition to the $\nu_{\text{NO}}$ stretch, $\nu_{\text{CN}}$ stretches at 2102, 2085 (sh), and 2072 cm$^{-1}$ confirm the Fe(CN)$_5$NO formulation.$^{22}$ Although there are no crystal structures of metal complexes of RSNO ligands, we note that related ligands that exhibit dominant O$^-$$-$$X$ resonance structures tend to be N-bound,$^{23}$ whereas ligands with dominant O$^-$$-$$N$ resonance structures are usually O-bound.$^{24}$ Given this precedent and the observed vibrational spectrum, we assign the molecular structure of the RP as $\text{[Fe(CN)$_5$}(\eta^1$$^-$$N$-RSNO)$]^{3-}$. Figure 2 illustrates the time-dependent IR spectra that are measured when the RP of NP and EtSH decomposes at $pD = 11$. The initial spectrum shows $\nu_{\text{CN}}$ (2102, 2085 (sh), and 2072 cm$^{-1}$) and $\nu_{\text{NO}}$ (1380 cm$^{-1}$) bands that are assigned to the RP. Bands at 2088 and 1648 cm$^{-1}$ due to NP$_1$$^-$ and 2038 cm$^{-1}$ due to ferrocyanide, [Fe(CN)$_6$]$^{4-}$, are observed as the RP decomposes (see ref 7). The ferrocyanide $\nu_{\text{CN}}$ band rapidly obliterates the weak band at 2048 cm$^{-1}$ present in the initial spectrum (labeled in Figure 1 but not in Figure 2) that corresponds to the nitro species [Fe(CN)$_5$(NO)$_2$]$^{4-}$, the product of competitive nucleophilic attack by OH$^-$. Note the principal reaction that is illustrated in Figure 2 is $\text{RP} \rightarrow \text{NP}_1$$^-$ (the molar absorptivity of ferrocyanide is an order

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photolytic decomposition.7,32 for the study of NP and the RP since these species are subject to the molecular structures of intermediates. We also note that IR present study using IR spectroscopy affords the first evidence of previously using primarily electronic and EPR spectroscopy. The of magnitude larger than that of NP1. The weak bands at 1773 and 1747 cm−1 are due to the ethyl ester of Roussin’s red salt, Fe2(SEt)2(NO)4,28 which we sometimes observed as a minor product during the reduction of NP under anaerobic conditions. Importantly, there is a 1:1 correspondence between the loss of the RP and the fate of the RP (apparently homolytic cleavage of the N–S bond of the RP to yield NP− and thyl radical) are consistent with a mechanism of NP-catalyzed oxidation of thiols to disulfides.29 Furthermore, NP reacts with enzymes (e.g., papain and glyceraldehyde phosphate dehydrogenase) that possess SH groups at their active sites to form disulfide bonds that lead to enzyme inhibition.30 Our observations concerning the structure and fate of the RP (apparently homolytic cleavage of the N=S bond of the RP to yield NP− and thyl radical) refer to a study: Wanner, M.; Scheiring, T.; Kaim, W.; Slep, L. D.; Baraldo, L.; Olabe, J. A.; Za´lis ˇ; Baerends, E. J. Inorg. Chim. Acta 2001, 40, 5704. (15) Enemark, J. H.; Feltham, R. D. Coord. Chem. Rev. 1974, 10, 55. (11) We obtained similar results using an IR-transparent thin-layer cell. (12) We have observed NP− converted to NP+ upon acidification of unbuffered (cf. Figure 1) solutions (and vice-a-versa). (13) This literature value for K1, determined by pulse radiolysis,7 is consistent with the spectra of Figure 1 and the observation (Supporting Information) that equimolar amounts of NP+ and NP− are observed at pH = 7.72 (pD = 7.40) for [NP−] = [NP+] = 600 μM (from which we calculate K1 = 7.22 × 10−6.) (14) In Nigel, [Fe(N3)4(NO)2](NO2)2: 2075 (vNO); 1568 (v≡N); [Fe(N3)(NO)]2: 2110 (vN3); 1792 (v≡N). See also the DFT study: Wanner, M.; Scheiring, T.; Kaim, W.; Slep, L. D.; Baraldo, L.; Olabe, J. A.; Za´lis ˇ; Baerends, E. J. Inorg. Chem. Acta 2001, 40, 5704. (16) Manoharan, P. T.; Williams, D. L. H. J. Chem. Soc., Dalton Trans. 2004, 3633. For further details see ref 14. (17) The RP has alternatively been described as [Fe(CN)5(NO)(SR)]−,[Fe(CN)5(NO)(SR)]2−, [Fe(CN)4(NO)(SR)]−,[Fe(CN)4(NO)(SR)]2−, and other formulations: Jaksicic-Milic, M.; Hankonyi, V.; Karas-Gasperec, V. Z. Phys. Chem. 1980, 261, 1041 and ref 7. (18) Manoharan, P. T.; Gray, H. J. J. Am. Chem. Soc. 1965, 87, 3340. (19) We have observed similar vRSO stretches when propanol was employed (1375 and 1355 cm−1 for 13NP and 35NP, respectively). Longer chain alkylthionates are considerably less soluble and their C–H bending modes begin to obscure the vRSO stretches. (20) The vRSO stretch of NP15 may be compared with the range of 1338–1394 cm−1 that has been computed for [Fe(CN)5(HNO)]− using DFT (González Lebrero, M. C.; Scherlis, D. A.; Estú, G. L.; Olabe, J. A.; Estrin, D. A. Inorg. Chem. 2001, 40, 4127) and the value of 1385 cm−1 that has been measured for MBHNO (Farmer, P. J., Immoos, C. E.; Bocian, D. F.; Czarnecki, K. Personal communication). (21) The calculated frequency for a 15NO harmonic oscillator is 1355 cm−1. (22) Williams, D. L. H. Acc. Chem. Res. 1999, 32, 869. (23) PM3 calculations suggest the HOMO of NP15S has substantial Feδ−NOφ character that indicates back-bonding. (24) Three IR CN stretches are generally observed for M(CN)X complexes, a symmetric cis-CN stretch ν(A1), an axial CN stretch ν(νA) (A1), and an intense asymmetric cis-CN stretch νC (E): Griffith, W. P.; Mockford, M. J.; Skapski, A. C. Inorg. Chem. Acta 1987, 126, 179. In general, νE > νA > νC, although the latter two bands often overlap. (25) See for example the CSD refcodes: BANW1, CMPBRU, CNSBRP, FAYCIO, FAVFIR, HSIUSD, HSIQAF, HSIUFE, HISFY. For further details see ref 14. (26) See for example the CSD refcodes: BOXSON, BUYTAQ, HISOCS, MNACUC10, TEMFAS, YUFICL, ZAWBAA. (27) Swinehart, J. H.; Rock, P. A. Inorg. Chem. 1966, 5, 573. (28) Rauchfuss, T. B.; Weatherill, T. D. Inorg. Chem. 1982, 21, 827. (29) Morando, Pedro J.; Borghu, Elena B.; De Schneitgest, Lydia M.; Blesa, Miguel A. J. Am. Chem. Soc., Dalton Trans. 1981, 435. (30) Butler, A. R.; Csaly, A. M.; Johnson, I. L. Polyhedron 1990, 9, 913. (31) Butler, A. R.; Clay-Harrison, A. M.; Glidewell, C.; Sreenan, P. E. Polyhedron 1988, 7, 1197. (32) Szczawiński, K.; Oszajca, J.; Stoehel, G.; Stasicka, Z. J. Chem. Soc., Dalton Trans. 1999, 2353. (33) We note our experiments that involve the RP were carried out at high pH. We have not observed free RSNO in any of these experiments; however, we cannot address claims that a decomposition pathway exists for the RP between pH 6.5–8.5 that involves dissociation of RSNO (see Figure 3 of ref 6).