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Synthesis and Characterization of a Copper Complex of $[Et_2N(N_2O_2)]^-$ Stabilized with Respect to NO Release in Aqueous Solution

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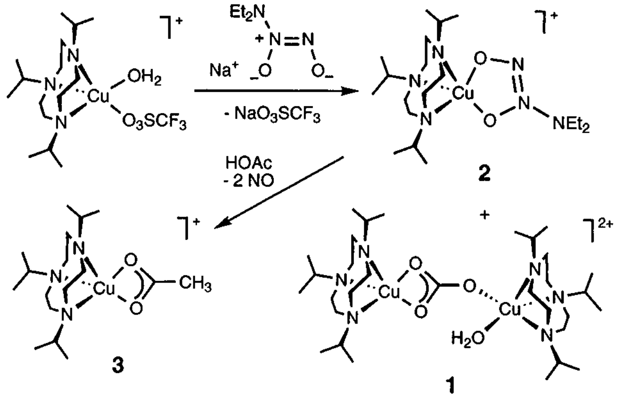
The wide-ranging biological effects and potential pharmacological implications of nitric oxide (NO)[1] have stimulated interest in developing prodrugs that evolve NO in a controlled manner in aqueous solution.[2] Important examples are the $[R_2N(N_2O_2)]^-$ ions (NONOates),[3,4] which have been shown to regenerate 2 equiv of bioactive NO spontaneously in H_2O at rates that depend on pH and the nature of the R groups.[5] In one strategy to further control NO release rates and increase target specificity, derivatives of these ions were prepared in which the oxygen atoms were alkylated[6] or metalated.[7,8] Of note with regard to the latter are several structurally characterized copper complexes of $[Et_2N(N_2O_2)]^-$, either homoleptic or having solvent coligands, that are stable in nonaqueous solvents.[7] However, these typically polynuclear complexes are reported to decompose in H_2O at the same rate as the parent sodium salt, implicating rapid displacement of the NONOate from the metal ion and subsequent NO release via hydrolysis of the free ligand. We hypothesized that, by using an appropriate coligand(s), copper complexes of NONOates with increased stability and, thus, greater potential as prodrugs for controlled and selective NO release might be accessible. We report that the sterically hindered 1,4,7-triisopropyl-1,4,7-triazacyclononane (L^{iPr3})[9] enables the preparation of a unique mononuclear copper complex of $[Et_2N(N_2O_2)]^-$ that exhibits enhanced stability in aqueous solution at 37 °C but which can be induced to rapidly evolve NO through the addition of acid or thiols.

Scheme 1



Admixture of solutions of $[L^{iPr3}Cu(H_2O)(O_3SCF_3)]O_3SCF_3$[10] and $Na[Et_2N(N_2O_2)]$ (1.8 equiv) in MeOH at −35 °C afforded a deep blue-green solution and a green precipitate after 30 min. The precipitate (obtained in low and variable yields) was identified as the carbonate complex $[(L^{iPr3}Cu)_2(\mu\text{-}\eta^1\text{:}\eta^2\text{-}CO_3)(H_2O)](O_3SCF_3)_2$, **1** (Scheme 1), by comparison of its UV−vis and IR spectra to those of independently synthesized material that was structurally characterized by X-ray crystallography.[11] Removal of solvent from the blue-green solution and crystallization from acetone/Et_2O at −20 °C yielded $\{L^{iPr3}Cu[Et_2N(N_2O_2)]\}O_3SCF_3$, **2**, in 49% yield after 3−5 days.[12] Close inspection revealed that the product consisted of a mixture of crystals of two different colors, blue and blue-green. Separate X-ray structure determinations on a crystal of each type revealed them to be slightly different morphological forms of the same

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(11) Complex **1** was independently prepared by allowing an aqueous solution of $[L^{iPr3}Cu(H_2O)(O_3SCF_3)]O_3SCF_3$ (0.210 g, 0.330 mmol, in ~3 mL) and 1.0 M NaOH (~0.5 mL) to stand in air (the presumed source of CO_2 for the CO_3^{2-} ligand). Green blocklike crystals of 1 deposited after 1 week (15 mg, 9%): mp 184−185 °C; UV−vis [CH_2Cl_2, nm (ϵ/complex, M^{-1} cm^{-1})] 304 (10 800), 388 (2300), 650 (350); FTIR (KBr) 2974, 1539, 1476, 1377, 1278, 1159, 1068, 1032, 962, 765, 720, 639, 570, 519 cm^{-1}; electrospray MS *m/e* (relative intensity) 847 ($[M - H_2O - O_3SCF_3]^+$, 100), 348 ($[L^{iPr3}CuOC]^+$, 74), 256 ($[L^{iPr3}]^+$, 63). Anal. Calcd for $C_{33}H_{68}Cu_2F_6N_6O_{10}S_2$: C, 39.08; H, 6.76; N, 8.29. Found: C, 39.25; H, 6.79; N, 8.32. Crystal data: $M_r = 1014.1$, monoclinic, space group $P2_1/c$, $a = 16.7355(4)$ Å, $b = 21.1711(5)$ Å, $c = 13.3955(3)$ Å, $\beta = 104.441(1)°$, $V = 4596.2(2)$ Å^3, $Z = 4$, $2\theta_{max} = 46.52°$, $\rho_{calc} = 1.466$ g cm^{-3}, $T = 173$ K. Data were collected using a Siemens SMART system (Mo Kα irradiation), and the structure was solved by direct methods. Full-matrix least-squares refinement on F^2 using SHELXTL V5.0 converged with R1 = 0.0441 and wR2 = 0.0916 for 6539 independent reflections with $I > 2\sigma(I)$ and 616 parameters. See the supporting information for a drawing of the structure and listings of structural parameters.

S0020-1669(96)00414-4 CCC: $12.00

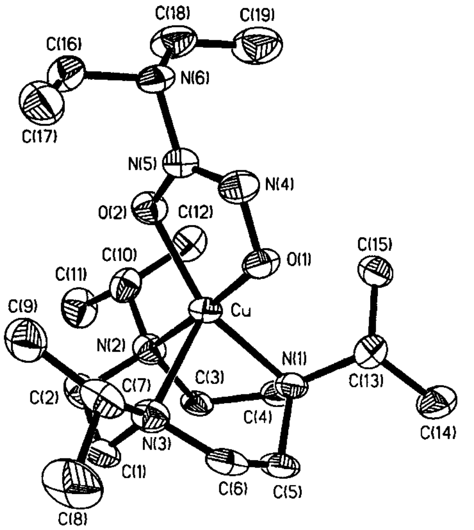

Figure 1. Representation of the cation in the X-ray crystal structure of the blue form of $\{L^{iPr3}Cu[\eta^2\text{-}Et_2N(N_2O_2)O,O]\}O_3SCF_3$, **2** (50% thermal ellipsoids; all hydrogen atoms omitted for clarity). Selected bond distances (Å) and angles (deg): Cu−O(1), 1.974(4); Cu−O(2), 1.980(4); Cu−N(1), 2.061(4); Cu−N(2), 2.024(5); Cu−N(3), 2.235(5); O(1)−N(4), 1.302(6); O(2)−N(5), 1.317(6); N(4)−N(5), 1.277(7); N(5)−N(6), 1.417(7); O(1)−Cu−O(2), 79.1(2); O(1)−Cu−N(2), 176.6(2); O(2)−Cu−N(2), 97.8(2); O(1)−Cu−N(1), 96.7; O(2)−Cu−N(1), 158.0(2); O(1)−Cu−N(3), 94.3(2); O(2)−Cu−N(3), 115.2(2); N(5)−N(4)−O(1), 112.8(4); N(4)−N(5)−O(2), 123.9(5); N(4)−N(5)−N(6), 116.3(5); N(4)−O(1)−Cu, 115.2(3); N(5)−O(2)−Cu, 108.7(3).

compound; a view of the molecule in the blue crystal is shown in Figure 1.[13] As in other complexes of analogous higher order nitrogen oxides,[7,8] the $[Et_2N(N_2O_2)]^-$ anion coordinates to copper via both oxygen atoms [Cu−O(1) = 1.974(4) Å, Cu−O(2) = 1.980(4) Å]. The blue and blue-green crystalline forms have almost identical molecular structures, with only slight differences apparent in their metal ion geometries; both are best described as distorted square pyramidal, but with minor divergences in interligand bond angles that are reflected in their τ values ($\tau = 0.31$ for the blue crystal versus $\tau = 0.48$ for the blue-green crystal, where $\tau = 0$ or 1.0 for idealized square pyramidal or trigonal bipyramidal geometries, respectively).[14]

Although **2** is stable for long periods at ambient temperature as a crystalline solid or in deionized aqueous solution at pH >7, rapid evolution of NO (∼80% yield based on a maximum of 2 equiv/complex) occurs upon addition of protonic acids (HCl, HOAc) or thiols (EtSH, C_6F_5SH). The copper-containing coproduct in the case when HOAc was used was identified as $[L^{iPr3}Cu(OAc)]O_3SCF_3$ (**3**), the X-ray crystal structure of which revealed η^2-binding of the acetate anion to a square pyramidal Cu(II) center.[15] To compare the rates of NO release from **2** and $Na[Et_2N(N_2O_2)]$, NO evolution from solutions of these species was monitored as a function of time using chemiluminescence detection.[16] Pseudo-first-order rate constants for the decay of **2** and the parent NONOate salt at 37 °C were calculated from the slopes of linear plots of $\ln[(A_\infty - A_t)/(A_\infty - A_0)]$ vs time (s) from data obtained over 3−4 half-lives (A = area under chemiluminescence response curve in mV·s; see Supporting Information). The observed rate constants were essentially identical in 0.1 M HCl [$k(\mathbf{2}) = k(\text{NONOate}) = 4.3(4) \times 10^{-2}$ s^{-1}], but **2** decomposed half as fast in 0.1 M HOAc [$k(\mathbf{2}) = 2.2(3) \times 10^{-2}$ s^{-1}, $k(\text{NONOate}) = 5.3(5) \times 10^{-2}$ s^{-1}] or PBS buffer (pH 7.4)[17] [$k(\mathbf{2}) = 1.2(1) \times 10^{-3}$ s^{-1}, $k(\text{NONOate}) = 2.8(4) \times 10^{-3}$ s^{-1}]. This divergence in NO evolution rates, although not large, nonetheless verifies the enhanced stability of **2**. In addition, it implies a decomposition mechanism for **2** different from that postulated for other copper complexes of $[Et_2N(N_2O_2)]^-$,[7] which evolve NO in H_2O via rapid ligand dissociation and subsequent hydrolysis at the same rate as the parent sodium salt. Future research will focus on unraveling the mechanism of decomposition of **2** and on developing even more "tunable" NO-releasing prodrugs through strategic derivatizations of the NONOate and the supporting triazacyclononane ligands.

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Supporting Information Available: Experimental procedures for the synthesis of all compounds and for the kinetics experiments, plus full details of the X-ray structures of **1**, the two forms of **2**, and **3**, including structural diagrams and tables of atomic coordinates, bond lengths, bond angles, and anisotropic thermal parameters (57 pages). Ordering information is given on any current masthead page.

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(12) Characterization data for **2**: mp 122−127 °C dec; UV−vis [MeOH, nm (ϵ/complex, M^{-1} cm^{-1})] 240 (4060), 278 (4880), 648 (100); FTIR (KBr) 2974, 1496, 1468, 1384, 1265, 1229, 1152, 1075, 1032, 970, 639, 519 cm^{-1}; EPR (MeOH, 9.43 GHz, 77 K) $g_\| = 2.23$, $g_\perp = 2.05$, $A_\|^{Cu} = 171 \times 10^{-4}$ cm^{-1}; cyclic voltammetry (0.2 M Bu_4NPF_6 in CH_2Cl_2) $E_{1/2} = -0.78$ V vs SCE, ΔE_p ranging from 110 mV at scan rate 20 mV s^{-1} to 250 mV at scan rate 500 mV s^{-1} (quasireversible behavior); electrospray MS m/e (relative intensity) 450 ($[M - O_3SCF_3]^+$, 100). Anal. Calcd for $C_{20}H_{43}CuF_3N_6O_5S$: C, 40.02; H, 7.22; N, 14.00. Found: C, 40.14; H, 7.17; N, 13.80.

(13) Crystal data for blue form of **2**: $C_{20}H_{43}CuF_3N_6O_5S$, $M_r = 600.2$, monoclinic, space group Cc, $a = 12.9638(9)$ Å, $b = 28.061(2)$ Å, $c = 8.9906(5)$ Å, $\beta = 121.567(1)°$, $V = 2786.7(3)$ Å^3, $Z = 4$, $2\theta_{max} = 46.52°$, $\rho_{calc} = 1.431$ g cm^{-3}, $T = 173$ K. Data were collected using a Siemens SMART system (Mo Kα irradiation), and the structure was solved by direct methods. Full-matrix least-squares refinement on F^2 using SHELXTL V5.0 converged with R1 = 0.0327 and wR2 = 0.0921 for 1946 independent reflections with $I > 2\sigma(I)$ and 368 parameters. Crystal data for blue-green form of **2**: $C_{20}H_{43}CuF_3N_6O_5S$, $M_r = 600.2$, monoclinic, space group $P2_1/c$, $a = 11.2541(2)$ Å, $b = 13.9901(1)$ Å, $c = 17.7935(1)$ Å, $\beta = 94.411(1)°$, $V = 2793.22(6)$ Å^3, $Z = 4$, $2\theta_{max} = 48.22°$, $\rho_{calc} = 1.427$ g cm^{-3}, $T = 173$ K. Data were collected using a Siemens SMART system (Mo Kα irradiation), and the structure was solved by direct methods. Full-matrix least-squares refinement on F^2 using SHELXTL V5.0 converged with R1= 0.0452 and wR2 = 0.1177 for 4340 independent reflections with $I > 2\sigma(I)$ and 376 parameters.

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(15) Characterization data for **3**: UV−vis [MeOH, nm (ϵ/complex, M^{-1} cm^{-1})] 302 (5000), 672 (100); FTIR (KBr) 2980, 1637, 1517, 1496, 1468, 1398, 1274, 1258, 1223, 1155, 1068, 1031, 967, 689, 637 cm^{-1}; electrospray MS m/e (relative intensity) 377 ($[M - O_3SCF_3]^+$, 100). Crystal data: $C_{18}H_{33}CuF_3N_3O_5S$, $M_r = 524.07$, monoclinic, space group $P2_1/c$, $a = 8.7036(1)$ Å, $b = 22.7080(1)$ Å, $c = 12.3177(2)$ Å, $\beta = 99.194(1)°$, $V = 2403.21(5)$ Å^3, $Z = 4$, $2\theta_{max} = 48.36°$, $\rho_{calc} = 1.448$ g cm^{-3}, $T = 173$ K. Data were collected using a Siemens SMART system (Mo Kα irradiation), and the structure was solved by direct methods. Full-matrix least-squares refinement on F^2 using SHELXTL V5.0 converged with R1 = 0.0449 and wR2 = 0.1202 for 3722 independent reflections with $I > 2\sigma(I)$ and 319 parameters. See the Supporting Information for a drawing of the structure and listings of structural parameters.

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(17) PBS buffer = 0.27 M KCl, 0.014 M NaCl, 1.1 mM Na_2HPO_4, and 0.15 mM KH_2PO_4.