

Characterization of 3-Aminophenazin-2-ol Isolated from the Chemical Oxidation of *o*-Phenylenediamine

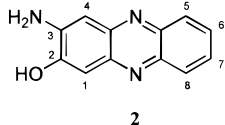
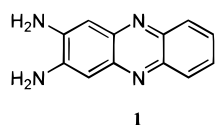
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Introduction

Phenazine-containing nonradioactive probes that explore nucleic acid structure have attracted considerable interest recently.¹ *o*-Aminohydroxy-containing compounds are also important ligands in transition metal chemistry that form complexes used as pH indicators.² Here we report the isolation of 3-aminophenazin-2-ol in good yield, which combines both phenazine and *o*-aminohydroxy moieties, that can be exploited to create metal complexes with novel chemical properties. Advantages include (a) the synthesis of DNA probes that vary the net charge of the complex, which is important in the exploration of the electrostatic interactions of molecules with nucleic acid helices, and (b) the creation of new indicator molecules that are sensitive to solvation or protonation due to the presence of basic phenazine nitrogens.



Mixtures of 2,3-phenazinediamine, **1**, and 3-aminophenazin-2-ol, **2**, have been produced in acidic media by the chemical oxidation of *o*-phenylenediamine (OPD) with ferric chloride³ and by electrochemical reduction of *o*-dinitrobenzene⁴ or *o*-dinitrosobenzene and the dioxime of 3,5-cyclohexadiene-1,2-dione.⁵ 3-Aminophenazin-2-ol has also been detected in trace quantities by the ammonium peroxydisulfate oxidation of OPD in acetic acid.⁶ Oxidation of OPD to the diimine, followed by condensation with a second molecule of OPD, yields **1** which is accompanied by partial hydrolysis to yield **2**. The proportion of **1** to **2** is dependent on the acid strength in the ferric chloride oxidation case, although the amount of **2** detected is low. We have extended this result to the chemical oxidation of OPD in strong acid with ammonium

peroxydisulfate with significant increase in the yield of **2** and report the accompanying NMR and crystallographic information.

Results and Discussion

The addition of ammonium peroxydisulfate to OPD in 1.0 M HCl yields a large crop of black crystals. The black crystals are the hydrochloride salts of a mixture of amino- and hydroxy-substituted phenazine derivatives. Elemental analyses and titration with strong base yield 0.95 ± 0.1 equiv of HCl per phenazine, based upon the formula of amino- and hydroxy-substituted phenazine·HCl·2H₂O. Under alkaline conditions this black material is neutralized, and a separation can be achieved by extraction with organic solvent. Unreacted OPD was recovered as 2,3-dihydro-2,2,4-trimethyl-(1*H*)-1,5-benzodiazepine after chromatographing with acetone on alumina and confirmed by melting point,¹⁴ GC/MS, ¹H NMR,¹¹ and elemental analyses.

The fraction obtained in the organic layer is identified as 2,3-phenazinediamine, **1**. The ¹H NMR spectrum of this product is identical with material obtained from an independent synthesis.⁷ After neutralizing the aqueous layer and collecting the resulting precipitate, 3-aminophenazin-2-ol, **2**, is isolated. The singlet resonance at 6.89 ppm in **1**, attributed to the 1 and 4 aromatic protons, has been split into two peaks in **2** (6.92 and 7.16 ppm), indicating the molecule is not symmetric. An additional resonance at 11.3 ppm appears, attributed to the hydroxyl proton. ¹H NMR of *o*-aminophenol shows a similar chemical shift for the OH proton. The singlet at 6.92 ppm is assigned to the proton adjacent to the amine, and the resonance at 7.16 ppm is assigned to the proton adjacent to the hydroxyl group due to similarity in chemical shifts of the identically substituted derivatives. (The 1,4-proton resonance appears at 6.89 ppm in 2,3-phenazinediamine and at 7.25 ppm in 2,3-phenazinediol.) Elemental analyses and mass spectrometry also support the identification of **2**. To our knowledge, **2** has only been previously isolated in small amounts under different experimental conditions.

The composition of the original black crystals, then, is a mixture of protonated **1** and **2**. Figure 1A is the ¹H NMR spectrum of this material in MeOH-*d*₄. The NMR spectra of purified, protonated **1** and **2** are shown as well, which reveals the individual contributions of the two components. The symmetry of the chemical shifts of protonated **1** (Figure 1C) suggests the proton here has been chelated by the two terminal amine groups and does not protonate the less basic phenazine nitrogens. For **2**, it is likely that the basic amine remains protonated; however, the molecule is unsymmetric, resulting in a more complex spectrum than for protonated **2**. In Figure 2, the simulated NMR spectrum is compared to the aromatic proton region of **2**. The multiplet at 7.65 ppm in Figure 2B is somewhat complicated by overlap from residual proton resonances of **1**. Chemical shift and coupling constant data for protonated **2** are given in Table 1. Similar NMR parameters for neutralized **1** and **2** in DMSO-*d*₆ are given in the Experimental Section.

By integration of the singlets in Figure 1A, the relative proportion of **1** and **2** can be determined. Taking into

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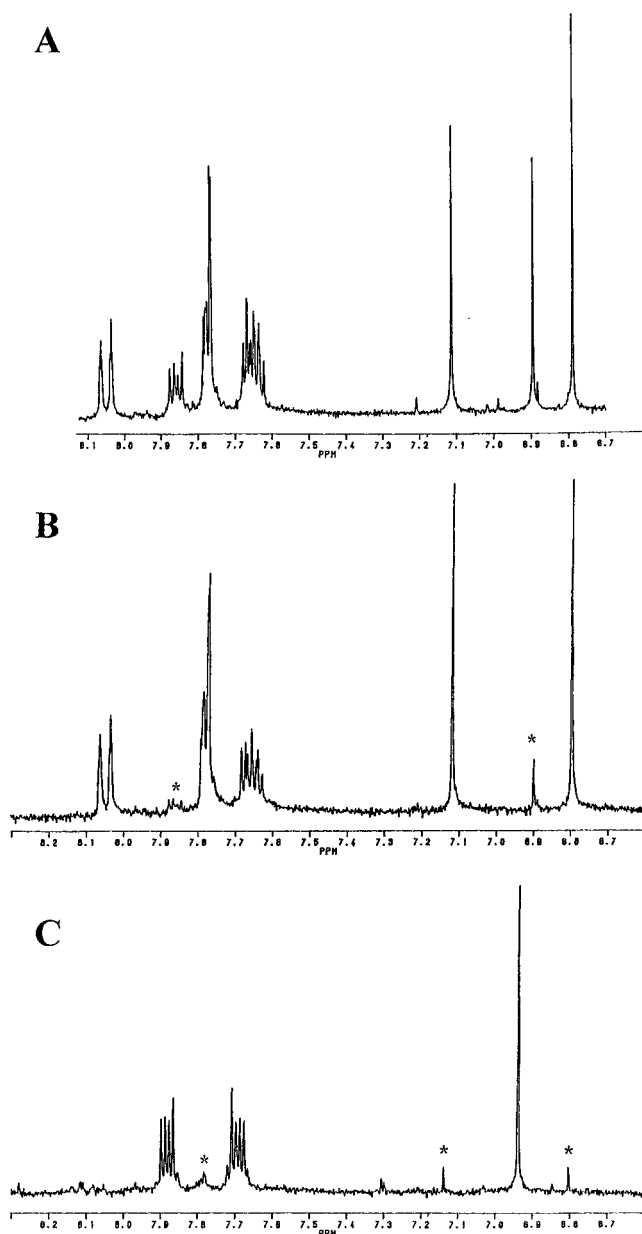


Figure 1. ^1H NMR spectrum (300 MHz) of (A) the black crystalline product produced by the ammonium persulfate oxidation of *o*-phenylenediamine, (B) protonated 3-aminophenazin-2-ol, **2** (asterisks indicate a slight presence of 2,3-phenazinediamine, **1**), and (C) protonated 2,3-phenazinediamine with 3-aminophenazin-2-ol impurity indicated by asterisks. All spectra were taken in $\text{MeOH-}d_4$.

account that the resonance at 6.94 ppm accounts for two ring protons in protonated **1**, we have found that the black crystalline material produced in this reaction is approximately 30% **1** and 70% **2** \pm 5%, based on repeated, independent syntheses. The elemental analyses of this material support this result.

The structure of the black crystalline material consists of protonated, cocrystallized diamino and aminohydroxy phenazine cations, a chloride anion, and approximately two water molecules.¹³ The position of the proton was not found, and there is substantial chloride and water disorder, plus a superposition of diamino and aminohydroxy molecular positions. Here, the diffraction experiment only confirms the topology of the molecular skeleton, which, in this case, is quite similar to the structure of protonated 2,3-phenazinediamine.⁸ Crystals

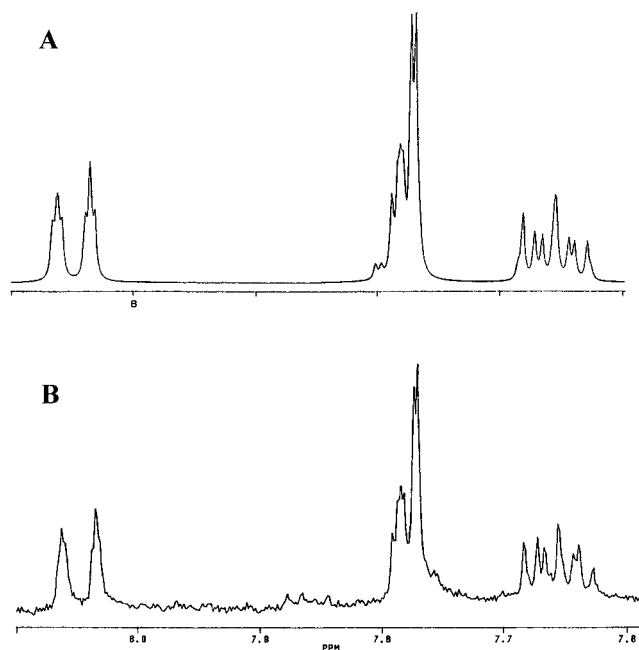


Figure 2. (A) Simulated NMR spectra of protonated **2** using a line broadening of 1.0 Hz and the spectral parameters reported in Table 1. (B) ^1H NMR spectrum (300 MHz) of the aromatic region of protonated **2** in $\text{MeOH-}d_4$.

Table 1. ^1H NMR Data for Protonated **2** in $\text{MeOH-}d_4$

	proton no.	chem shift (ppm)	coupling constants (Hz)
protonated 2	1	6.796	
	4	7.118	
	5	8.048	$J_{5-6} = 8.2$, $J_{5-7} = 1.5$, $J_{5-8} = 0.8$
	6	7.656	$J_{6-7} = 6.0$, $J_{6-8} = 1.5$
	7	7.779	$J_{7-8} = 4.0$
	8	7.773	

Table 2. Emission Maxima of Compounds **1** and **2** in Various Solvents

solvent	λ_{max} (nm)	
	1	2
dichloromethane	505	487
acetone	526	527
dimethyl sulfoxide	533	529
methanol	541	539

of purified **2** gave inferior crystallographic data, although the space group and cell constants remained the same as those reported here, indicating that the presence of **2** (70%) in the mixed crystal regulates the unit cell.

Fluorescence emission maxima are nearly identical for both **1** and **2** and exhibit a pronounced red shift ($\Delta\lambda \sim 50$ nm) with increasing solvent polarity (Table 2), typical of polar solutes, where the dipole moment in the excited state is larger than in its ground state, and the rotational relaxation time for the solvent molecules (10^{-12} – 10^{-10} s) is faster than the relaxation time of the excited singlet state of the molecule.⁹ For **2**, at room temperature, the excited state lifetime in acetone was measured as $\leq 10^{-8}$ s. In dichloromethane, **2** is practically insoluble, and the emission maximum is dependent on the amount of water

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present in the solvent. In dry CH_2Cl_2 , a new peak at 520 nm appears which we attribute to formation of the keto tautomer, 3-aminophenazin-2(10*H*)-one, commonly observed with other hydroxyphenazines.¹⁵

Conclusions

Previous reports describe the synthesis of 2,3-phenazinediamine⁷ and 2,3-phenazinediol¹² in high yield by combining OPD with 1,4-benzoquinone or 2,5-dihydroxy-1,4-benzoquinone, respectively. We have completed this sequence by isolating and characterizing the mixed member of the series, 3-aminophenazin-2-ol, in good yield. Synthesis and isolation are straightforward and ¹H NMR shows conclusively the relative proportion of products produced and the locations of protonation. We are currently pursuing the copper, cobalt, and ruthenium transition-metal coordination chemistry of this phenazine series.

Experimental Section

Elemental analyses were conducted by MHW Laboratories, Phoenix, AZ. Chemical shifts are referenced to the residual proton resonances of the solvent. NMR simulations were conducted using RACCOON, a PC-based modeling program.¹⁰ OPD (JT Baker, Lot 943360, mp 101–103 °C) was used without further purification.

OPD, 5.0 g (0.046 mol), was dissolved in 200 mL of 1.0 M HCl. To this solution was added 5.0 g (0.022 mol) of ammonium peroxydisulfate, producing a homogenous solution after stirring. The reaction mixture was allowed to stand at room temperature for 48 h. Filtration yielded ~1 g of black needles. Anal. Calcd for 30% **1** ($\text{C}_{12}\text{H}_{10}\text{N}_4\cdot\text{HCl}\cdot 2\text{H}_2\text{O}$) + 70% **2** ($\text{C}_{12}\text{H}_9\text{N}_3\text{O}\cdot\text{HCl}\cdot 2\text{H}_2\text{O}$): C, 50.85; H, 5.09; N, 16.30; Cl, 12.51. Found: C, 49.45; H, 4.63; N, 15.50; Cl, 13.54.

Separation of **1** and **2** was completed by dissolving the black needles in 150 mL of 2 M NaOH, filtering any undissolved solid,

and extracting the aqueous solution six times with 50 mL portions of dichloromethane. The aqueous layer was acidified to pH ~7, and the precipitated solid **2** was filtered and washed with distilled water. A sharp, small shoulder at 6.89 ppm in the proton spectrum indicates that a minute quantity of 2,3-diaminophenazine is still present.

2,3-Phenazinediamine, 1: mp >300 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 6.24 (4H, br, -NH₂), 6.89 (2H, s, 1,4-H), 7.53 (2H, m, 6,7-H), 7.87 (2H, m, 5,8-H); MS *m/z* calcd 210.24, found 210.

3-Aminophenazin-2-ol, 2: yield 0.40–0.46 g (~77%, based on ~1 g of isolated crystalline material); mp >300 °C; ¹H NMR (300 MHz, DMSO-*d*₆) δ 6.30 (2H, br, -NH₂), 6.92 (1H, s, 1-H), 7.16 (1H, s, 4-H), 7.59 (2H, m, 6,7-H), 7.92 (2H, m, 5,8-H), 11.30 (1H, br, -OH), 3.35 (s, water of hydration); MS *m/z* calcd 211.22, found 211. Anal. Calcd for $\text{C}_{12}\text{H}_9\text{N}_3\text{O}\cdot 0.5\text{H}_2\text{O}$: C, 65.45; H, 4.58; N, 19.08. Found: C, 65.68; H, 4.74; N, 18.73.

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(13) For $[(\text{C}_{12}\text{H}_9\text{N}_3\text{O})_{0.7}(\text{C}_{12}\text{H}_{10}\text{N}_4)_{0.3}]\cdot\text{Cl}\cdot 1.8\text{H}_2\text{O}$: space group = *P* $\bar{1}$; *a* = 6.723(2) Å, *b* = 9.828(1) Å, *c* = 11.105(2) Å; *Z* = 2; 1287 reflections with *I* ≥ 1.5σ(*I*), 184 parameters, *R* = 0.075, *R*_w = 0.082. Cocrystallized **1** and **2** can be described as a solid solution where the 2- and 3-sites are crystallographically distinct, which cannot be transformed into one another by any operation of the space group, but peak heights and thermal parameters do not suggest that the OH substituent is concentrated at either site. The statistical superposition of 30% **1** and 70% **2** (two possible orientations) leaves a 65% nitrogen occupancy at both positions. Atomic coordinates, bond angles, bond lengths, and other crystallographic data have been submitted to the Cambridge Crystallographic Data Centre. This material can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EŽ, U.K.

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