corded (θ-2θ scans at 291 K) to θ_{max} = 28° (graphite-monochromated Mo K$\bar{\alpha}$ X-radiation, $\bar{\lambda}$ 0.71069 Å) on an Enraf-Nonius CAD4 diffractometer and were corrected empirically for absorption. The structure was solved by Patterson (Ni and I atoms) and difference Fourier techniques and refined by full-matrix least squares (SHELX[10]) to an R value of 0.0499, with a weighted index R_w ($= \sum w^{1/2}||F_o| - |F_c||/\sum w^{1/2}|F_o|$) of 0.0603 ($w^{-1} = \sigma^2(F_o) + 0.0076F_o^2$) for 3365 reflections with $F_o \geq 2.0\sigma(F_o)$. In the final stages of refinement, hydrogen atomic positions were idealized and all non-hydrogen atoms were allowed anisotropic thermal motion.[11]

Figure 2 shows the molecular structure of **1c** together with some important molecular parameters. The geometry at Ni is best described as square pyramidal (SP) with the Ni atom displaced ca. 0.34 Å out of the basal plane[12] toward the apical atom I1 although there is a significant distortion from an ideal SP geometry toward trigonal bypyramidal (TBP, C1 and I2 axial). As far as we are aware compound **1c** represents the first known example of a true organonickel(III) species. Ni-C1 is 1.898 (5) Å, and the average Ni-N bond is 2.044 (4) Å, these bond lengths in **1c** being consistently ca. 0.07 Å longer than equivalent distances[13] in the four-coordinate Ni(II) complex Ni[$C_6H_3(CH_2NMe_2)_2$-o,o']OC(O)H. However, the significance of this observation is difficult to assess since both the oxidation state of the metal and its coordination number change between these species in ways that would be expected to alter bond lengths in opposite senses. From the average Ni-I distance of 2.62 Å an approximate covalent radius for five-coordinate Ni(III) of ca. 1.3 Å may be estimated and compared with the value of ca. 1.2 Å calculated from the non-Jahn-Teller-elongated Ni-Br bonds of $NiBr_3(PPhMe_2)_2$.[14]

The crystallographic study now provides a rationalization of the ESR results that can be seen to be consistent with a Ni(III) low-spin d^7 system $[(e)^4(b_2)^2(a_1)^1]$ in which the unpaired electron is in an orbital of d_{z^2} symmetry, i.e., the orbital containing the apical halide.[15] Moreover, EHMO calculations[11] indicate that the unpaired electron resides primarily in a molecular orbital that is antibonding between Ni d_{z^2} and I1 p_z. The stability of these neutral 17-electron species is interesting since dimerization could theoretically provide an 18-electron configuration. It is too early to say whether the stability of the monomeric unit is steric or electronic in origin, but it should be noted that the proximal methyl functions $(C9)H_3$ and $(C11)H_3$, which stand axially to the two five-membered mirror-plane-related $\overline{NiCCCN}$ rings, serve to protect the vacant coordination site trans to I1.[16]

The formation of the five-coordinate d^7 **1a-c** from the d^8 species **2** can be considered as a ligand-transfer oxidation reaction, and cyclovoltametric measurements show that an electrochemically irreversible single-electron oxidation of d^8 **2b** to d^7 **1b** occurs at +0.38 V.[17] The existence of the SP Pt(II) complex Pt[C_6H_3-$(CH_2NMe_2)_2$-o,o'](RNC(H)NR)HgClBr (R = p-tolyl)[18] suggests that the path of the oxidation reaction of the SP Ni(II) complexes **2a** and **2b** with CuX_2 may involve a heterobimetallic intermediate having a related structure. Noteworthy is the fact that the carbanionic terdentate ligand used in these studies also assists the oxidation of other metal centers. The Pt(IV) complexes Pt-[$C_6H_3(CH_2NMe_2)_2$-o,o']X_3 may be prepared in a way similar to **1a-c** from appropriate Pt(II) materials.[19] Further studies on the properties of these Ni(III) and Pt(IV) species as well as the ability of the terdentate ligand to stabilize higher oxidation states are in progress.

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Registry No. 1a, 84500-90-3; **1b**, 84520-52-5; **1c**, 84500-91-4; **2a**, 84500-92-5; **2b**, 84500-93-6; **2c**, 84500-94-7; $CuCl_2$, 7447-39-4; $CuBr_2$, 7789-45-9; I_2, 7553-56-2.

Supplementary Material Available: Atomic coordinates, anisotropic thermal parameters, interatomic distances, and bond angles (4 pages). Ordering information is given on any current masthead page.

(10) Sheldrick, G. M., University of Cambridge, **1976**.
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(16) The shortest mutual contact is H(93)···H(111) at 2.93 Å.
(17) Referenced to the standard H_2 electrode. The potential was measured at +0.24 V in acetone by using 0.1 M Bu_4NBr as base electrolyte with Pt electrodes referred to an Ag/AgCl (0.1 M LiCl in acetone) reference electrode. See: Bond, A. M.; Hendrickson, A. F.; Martin, R. L. *J. Am. Chem. Soc.* **1973**, *95*, 1449-1456.
(18) In this complex the heterobimetallic interaction is donative from Pt(II) to Hg(II) but nevertheless results in a similar terdentate ligand conformation and metal coordination geometry as that found in **1c**: van der Ploeg, A. F. M. J.; van Koten, G.; Vrieze, K.; Spek, A. L.; Duisenberg, A. J. M. *Organometallics* **1982**, *1*, 1366.
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(20) The small features observable at approximately 2915, 3270, and 3410 G are assignable to another Ni(III) complex, Ni[$C_6H_3(CH_2NMe_2)_2$-o,o']BrCl, which has been separately synthesized and characterized. Its presence was due to a contamination of a sample of **2a** by **2b**.

Formation of a σ-Bonded Aryliron Complex in the Reaction of Arylhydrazines with Hemoglobin and Myoglobin[1]

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Phenylhydrazine inactivates hemoglobin in vivo and triggers its precipitation in the form of Heinz bodies.[2] Myoglobin is similarly disabled by phenylhydrazine even though it does not precipitate from solution.[3] Stoichiometric studies establish that six molecules of phenylhydrazine are consumed and five molecules of benzene are formed per heme in these reactions. The sixth phenyl residue is found incorporated into *N*-phenylprotoporphyrin IX when the hemoproteins are denatured aerobically in the presence of acid, but only heme is recovered if denaturation occurs in the absence of oxygen.[3] We have proposed that the phenyl group in the inactivated hemoprotein is σ-bonded to the heme iron but undergoes an oxidative shift to the heme nitrogen as the protein denatures. This proposal is supported by a model for the iron-nitrogen shift,[4] although the available data do not definitively rule out either a phenyldiazene-iron complex[5] or a reversible *N*-phenylheme complex.[4b] We unambiguously demonstrate here that

(1) This research was supported by National Institutes of Health Grant AM 30297.
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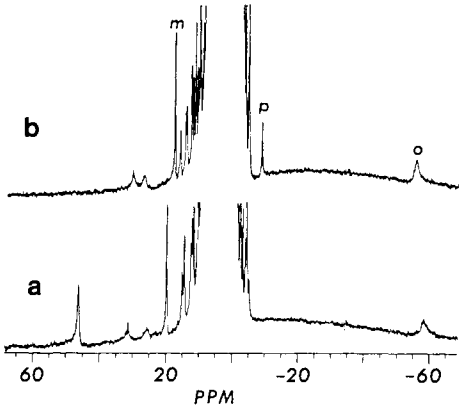

Figure 1. 240-MHz NMR spectra of the myoglobin complex formed by (a) reaction of the hemoprotein (6 mM) with *p*-tolylhydrazine and (b) reaction of myoglobin (4 mM) with phenylhydrazine. The iron aryl protons are labeled as follows: o, ortho; m, meta; p, para.

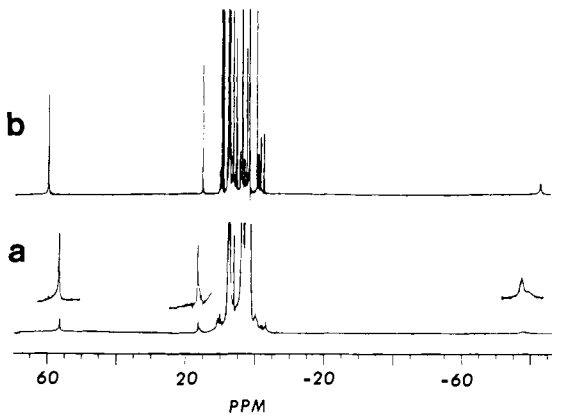

Figure 2. 240-MHz NMR spectra of the *p*-tolylheme complex (a) extracted from *p*-tolylhydrazine-treated hemoglobin (in acetone-d_6) and (b) from reaction of dimethyl esterified hemin with *p*-tolylmagnesium bromide (in deuterochloroform).

the aryl residue in the hemoproteins is indeed bound by a σ-bond to the prosthetic heme iron atom.

The 240-MHz NMR spectrum[6] of *p*-tolylhydrazine-inactivated myoglobin[7] exhibits peaks at 47, 20, and −58 ppm (3:2:2 ratio) distinctly outside the region occupied by the parent hemoprotein signals (Figure 1a). The three peaks are due to the *p*-methyl, meta, and ortho protons of the tolyl group, respectively. The phenyl meta, para, and ortho protons in the spectrum of phenylhydrazine-treated myoglobin (Figure 1b) appear at −55 (2 H), 18 (2 H), and 8 ppm (1 H). Addition of cyanide (40 mM) to the *p*-tolyl complex does not alter the NMR spectrum, evidence that the iron ligands prevent coordination of cyanide.[8]

The aryl–heme complex, although not removed from the hemoproteins by dialysis or column chromatography,[3] can be extracted intact with 2-butanone under argon in the presence of butylated hydroxytoluene (BHT).[9] The complex extracted from phenylhydrazine-inactivated hemoglobin exhibits a Soret band at 410 nm and a peak at 562 nm with a poorly resolved shoulder at approximately 530–540 nm, a spectral pattern distinct from that expected for iron *N*-phenylprotoporphyrin IX[10] but identical with that of the phenyliron complex from the reaction of phenylmagnesium bromide with the dimethyl ester of chloroiron protoporphyrin IX.[12] The aryl proton NMR chemical shifts of the synthetic and biological aryl–heme complexes are also identical except for slight differences (Figure 2) due to the fact that the spectra were recorded in different solvents. The importance of the sixth ligand and the environment on the NMR shifts of the aryliron protons is evident in the change that accompanies separation of the complex from the protein framework (Figures 1a and 2).

The magnitude of the aryl proton chemical shifts and the identity of the hemoprotein-derived complex with a synthetic sample establish that the reaction of arylhydrazines with hemoglobin or myoglobin results in formation of a σ-bond between a carbon of the aryl moiety and the prosthetic heme iron atom. Iron–carbon σ-bonds have been invoked to rationalize the high absorption maxima of complexes between cytochrome P-450 and certain substrates,[13] although the only experimental support for this postulate is the fact that synthetic model complexes absorb at comparable wavelengths.[14] Of particular relevance is the observation that a transient phenylhydrazine–cytochrome P-450 complex is the overture to irreversible inactivation of the enzyme.[15] The present direct identification of stable σ-bonded aryliron complexes in hemoglobin and myoglobin provides a firm precedent for the existence of similar complexes in other hemoproteins.

Acknowledgment. The assistance of Dr. David E. Kerr is gratefully acknowledged.

Registry No. *p*-Tolylhydrazine, 539-44-6; phenylhydrazine, 100-63-0; *p*-tolylheme complex, 84623-07-4.

(6) 240-MHz hemoprotein NMR spectra were determined at 22 °C in deuterated 100 mM phosphate buffer containing 1 mM EDTA. A sweep width of 50 KHz over 32K data points was used. A 4-Hz line broadening function was applied to each FID prior to transformation. The residual water proton signal at 4.61 ppm was suppressed by a 1-s presaturation pulse.

(7) Solutions of aryl hydrazine hydrochlorides (25 mM) and sperm whale skeletal muscle myoglobin (4–6 mM) in 100 mM deuterated phosphate buffer (pD 6.0, 1 mM EDTA) were agitated for 15 min and filtered before analysis by NMR. The deuterated buffer was prepared by adding KH_2PO_4, Na_2HPO_4, and EDTA to deuterated water, lyophilizing, and reconstituting to the required volume with 99.96% deuterated water.

(8) Coordination of cyanide to the iron would change its spin state and alter the NMR spectrum: La Mar, G. N.; Walker (Jensen), F. A. In "The Porphyrins"; Dolphin, D., Ed.; Academic Press: New York, 1979; Vol. 4, pp 61–157.

(9) Human type IV hemoglobin (Sigma, 4 mM in heme), aryl hydrazine hydrochloride (25 mM), and EDTA (1 mM) in 5 mL of deionized water were stirred at 25 °C for 15 min. The solution was then purged by gently bubbling argon through it for 20 min before 5 mL of argon-presaturated 2-butanone (dried over 3-Å molecular sieves) containing BHT (0.025%) was added. The mixture, shaken gently, was allowed to sit for 5 min. The absorption spectrum of an aliquot of the red organic layer was taken in tetrahydrofuran containing 0.025% BHT. The red organic layer was then transferred by syringe to a flask (argon atmosphere) placed in a −70 °C bath. The solvent was removed at −70 °C under low pressure (1 mmHg). The NMR spectrum of the residue was recorded in 1 mL of argon-saturated acetone-d_6 containing 0.025% BHT. The BHT *tert*-butyl methyl signal at 1.3 ppm was used as the reference.

(10) Insertion of iron into *N*-phenyltetraphenylporphyrin by the procedure of Lavallee[11] gives the iron complex with spectral properties (λ_{max} 452 nm, 466, 568, 630, 682) that clearly distinguish it from the iron–phenyltetraphenylporphyrin complex (λ_{max} 419 nm, 526).[4]

(11) Lavallee, D. K. *J. Inorg. Biochem.* **1982**, *16*, 135–142.

(12) Phenylmagnesium bromide or the Grignard reagent prepared from *p*-methylphenyl bromide (1.2 equiv) was added at −70 °C under argon to dimethyl esterified chloroiron protoporphyrin IX (100 mg) dissolved in 10 mL of dry tetrahydrofuran. The solution became red on warming to room temperature. The reaction progress was monitored by the disappearance of the parent porphyrin Soret band. As soon as the reaction was complete, BHT (20 mg) was added, and the solution was concentrated to 4 mL (rotary evaporator). The concentrate was chromatographed on basic alumina (activity I) with 1:1 hexane:tetrahydrofuran containing 0.025% BHT as the eluent. The red fraction was concentrated on a rotary evaporator, the residue taken up in 1 mL of dry peroxide-free tetrahydrofuran, 10 mL of hexane added, the the resulting mixture cooled to −70 °C and filtered. The red precipitate, washed with hexane, was dried under vacuum. The NMR spectrum was taken in deuterated chloroform containing 0.025% BHT.

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