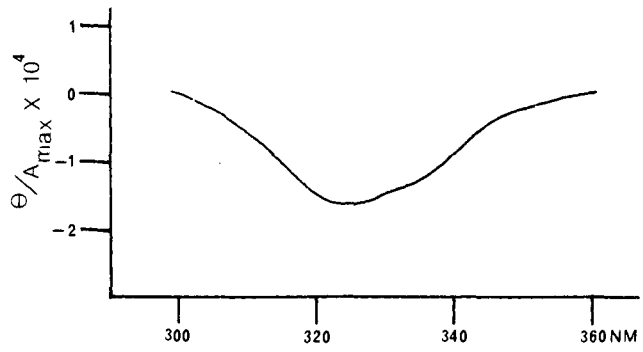


Figure 2. Circular dichroism curve of the pigment formed from 11,12-dihydroretinal **2** and bovine opsin in 1% ALO, pH 7.

hydride and subsequent oxidation with manganese dioxide. Separation by analytical HPLC, μ-Porasil, 10% ether in hexane, of the major isomer of the four-component mixture afforded pure *all-trans*-11,12-dihydroretinal (**2**) as characterized by ^{1}H NMR and ^{13}C NMR spectra. The chromophore is extremely unstable to traces of acid, base, or oxygen, and starts to deteriorate in a few days even when stored under nitrogen in the dark at −20 °C. Because of its lability, the chromophore should be kept as ester **5**, and converted to the aldehyde and purified by HPLC immediately prior to usage, or stored at −65 °C under argon.

Incubation of this dihydroretinal with bovine opsin suspension[8,9] for 3 h at 37 °C resulted in the appearance of a peak above 300 nm. The product was then purified by calcium phosphate chromatography, 1% Ammonyx LO.[10] Control incubations employing only opsin or the chromophore gave no peaks above 300 nm and hence the new peak at 345 nm (Table I, Figure 1), which was accompanied by a CD extremum (Figure 2),[11] must be due to pigment formation. As expected, the UV spectrum was changed neither by exposure to room light for 10 h nor by direct irradiation for 0.5 h at room temperature with a 275-W sun lamp, and hence the pigment is "nonbleachable".

Absorption data for the dihydro chromophores **2, 6, 7,** and pigment are shown in Table I together with the corresponding data for 11-*cis*-retinal and rhodopsin (see also Figure 1). The spectra of the protonated Schiff bases (SBH^+) were measured in the "leveling" solvents ethanol and methanol because in these solvents the UV spectra of SBH^+ are insensitive to the method of preparation and the counteranion.[12]

One of the most important unsolved problems in vision chemistry concerns the large red shifts seen in the various rhodopsins, which absorb from 460 to 560 nm depending on the opsin,[13] as opposed to the 440-nm value for SBH^+ (Table I). Numerous models and theoretical calculations have been forwarded to account for this. For example, the following electrostatic interactions between SBH^+ and the protein receptor site have been proposed: (i) between $C{=}N^+H$ and a nearby counterion;[14] (ii) between $C{=}N^+H$, a nearby anion, and an additional anion close to the trimethylcyclohexene ring;[15] (iii) between delocalized positive charge and nucleophilic groups along the side chain;[16] (iv) between $C{=}N^+H$ and polarizable aromatic amino acid residues,[17] etc.

However, it is remarkable that, in spite of the much shorter chromophore of dihydroretinal, the red shift between SBH^+ and pigment is *larger* than in the case of natural rhodopsin, i.e., 270 nm to 345 nm (or 8051 cm^{-1}) in contrast to 440 nm to 500 nm (or 2727 cm^{-1}) (Table I).

None of the above theories can satisfactorily ratonalize this dramatic shift of 8051 cm^{-1}. It is quite possible that several effects are contributing simultaneously; it is also possible that the situation encountered in the dihydro pigment differs from normal rhodopsins. However, there undoubtedly exists within the protein cavity an environment which induces the short dihydro-SBH^+ chromophore to undergo a further red shift of 75 nm. Preparations of other dihydroretinals,[18] comparisons of spectral properties of their pigments formed from bovine and other opsins, and theoretical calculations based on the results[19] will contribute to an understanding of this problem.[20]

References and Notes

(1) R. Hubbard and G. Wald, *J. Gen. Physiol.*, **36**, 269 (1952); G. Wald, *Science*, **162**, 230 (1968). Also see the following for a general account: *Acc. Chem. Res.*, **8**, 81–112 (1975).
(2) F. D. Callino, *Nature*, **171**, 469 (1953); R. A. Morton and R. A. J. Pitt, *Biochem. J.*, **59**, 128 (1955); A. R. Oseroff and R. H. Callender, *Biochemistry*, **13**, 4243 (1974); A. Lewis, R. S. Fager, and E. W. Abrahamson, *J. Raman Spectrosc.*, **1**, 465 (1973); R. Mathies, A. R. Oseroff, and L. Stryer, *Proc. Natl. Acad. Sci. U.S.A.*, **73**, 1 (1976); R. H. Callender, A. Doukas, R. Crouch, and K. Nakanishi, *Biochemistry*, **15**, 1621 (1976).
(3) D. Bownds, *Nature*, **216**, 1178 (1967); M. Akhtar, P. J. Blosse, and P. B. Dewhurst, *Biochem. J.*, **110**, 693 (1968); W. J. DeGrip, S. L. Bonting, and F. J. M. Daemen, *Biochem. Biophys. Acta*, **303**, 189 (1973); R. S. Fager, P. Sejnowski, and E. W. Abrahamson, *Biochem. Biophys. Res. Commun.*, **47**, 1244 (1973).
(4) T. Yoshizawa and Y. Kito, *Ann. Rep. Sci. Works, Fac. Sci. Osaka Univ.*, **6**, 27 (1958).
(5) G. E. Busch, M. L. Applebury, A. A. Lamola, and P. M. Rentzepis, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 2802 (1972); V. Sundstrom, P. M. Rentzepis, K. Peters, and M. L. Applebury, *Nature*, **267**, 645 (1977).
(6) T. G. Ebrey and T. Yoshizawa, *Exp. Eye Res.*, **17**, 545 (1973); T. Yoshizawa and S. Horiuchi in "Biochemistry and Physiology of Visual Pigments," H. Dartnall, Ed., Springer-Verlag, Berlin, 1973, p 146.
(7) W. K. Chan, K. Nakanishi, T. G. Ebrey, and B. Honig, *J. Am. Chem. Soc.*, **96**, 3642 (1974); K. Nakanishi, A. P. Yudd, R. K. Crouch, G. L. Olson, H.-C. Cheung, R. Govindjee, T. G. Ebrey, and D. J. Patel, *J. Am. Chem. Soc.*, **98**, 236 (1976), and references cited therein.
(8) D. S. Papermaster and W. J. Dreyer, *Biochemistry*, **13**, 2438 (1974).
(9) M. Zorn and S. Futterman, *J. Biol. Chem.*, **224**, 881 (1971).
(10) H. Shichi, M. Lewis, F. Irreverr, and A. Stone, *J. Biol. Chem.*, **224**, 529 (1969); T. Ebrey, *Vision Res.*, **11**, 1007 (1971); M. L. Applebury, D. M. Zuckerman, A. A. Lamola, and T. M. Jovin, *Biochemistry*, **13**, 3448 (1974).
(11) The optical activity can be accounted for if the negative charge were chirally situated with respect to the protonated Schiff base linkage. An additional factor which may be contributing to the optical activity is interaction between the enal chromophore and an aromatic amino acid moiety. With respect to the origin of the CD extrema of natural rhodopsin, an inherently chiral twist of the chromophore would probably be contributing as well.
(12) P. E. Blatz, K. H. Johnson, J. H. Mohler, S. K. Al-Dilaimi, S. Dewhurst, and J. O. Erickson, *Photochem. Photobiol.*, **13**, 237 (1971); P. E. Blatz, J. H. Mohler, and H. V. Navangul, *Biochemistry*, **11**, 848 (1972).
(13) B. Honig and T. G. Ebrey, *Ann. Rev. Biophys. Bioeng.*, **3**, 151 (1974).
(14) P. E. Blatz, J. H. Mohler, and H. V. Navangul, *Biochemistry*, **11**, 848 (1972); H. Suzuki, T. Komatsu, and T. Kato, *J. Phys. Soc. (Jpn.)*, **34**, 156 (1973).
(15) B. Honig, A. D. Greenberg, V. Dinur, and T. G. Ebrey, *Biochemistry*, **5**, 4593 (1976).
(16) A. Kropf and R. Hubbard, *Ann. N.Y. Acad. Sci.*, **74**, 266 (1958); M.-J. Mantione and B. Pullman, *Int. J. Quantum Chem.*, **5**, 349 (1971); W. H. Waddell, A. M. Schaffer, and R. S. Becker, private communication.
(17) C. S. Irving, G. W. Byers, and P. A. Leermakers, *Biochemistry*, 9, 858 (1970).
(18) Preparation of 7,8-, 9,10-, and 13,14-dihydroretinals and their pigments is in progress.
(19) Calculations are being carried out by Professor B. Honig, Hebrew University, Israel.

(20) The studies were supported by NIH Grants EY 01253, EY 01811, and a grant from Hoffmann-La Roche, Inc. We also acknowledge the help of Dr. F. Pilkiewicz and M. Pettei in the HPLC purification of the dihydroretinal.

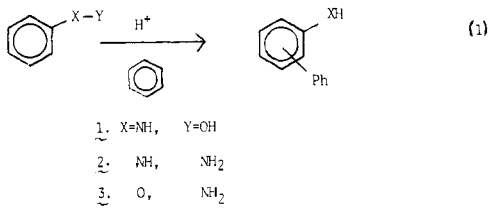
Mary Ann Gawinowicz, Valeria Balogh-Nair
Jeffrey S. Sabol, Koji Nakanishi*
Department of Chemistry, Columbia University
New York, New York 10027

Received July 11, 1977

A Novel Phenol–Benzene C–C Coupling Reaction. An Acid-Catalyzed Reaction of *N*-Acyl-*O*-arylhydroxylamines with Benzenes

Sir:

The reaction of *N*-phenylhydroxylamine[1,2] (**1**, X = NH; Y = OH), phenylhydrazine[3] (**2**, X = NH; Y = NH_2), and related compounds[3-5] with benzene give aminobiphenyls (eq 1) or their derivatives, and may involve a positively charged



(1)

1. X=NH, Y=OH
2. NH, NH_2
3. O, NH_2

nitrogen atom. The intermediacy of a phenoxenium ion with a positively charged oxygen atom has been considered in an oxidative coupling reaction of phenol.[6] In view of these, we have studied a reaction of *O*-phenylhydroxylamine (**3**, X = O; Y = NH_2) which is isoelectric with **1** and **2**, and their derivatives of **3** with benzene. A related example has been found in a thermolytic reaction of 4-nitrophenoxypyridinium salt with anisole reported by Abramovitch and coworkers.[7] Quite recently he also reported the formation of 4-nitrophenoxenium ion from an acyl derivative of 4-nitrophenylhydroxylamine.[8] In the present paper, we wish to describe the reaction of the simplest phenoxenium ion and benzene. In addition to the mechanistic discussion, a synthetic application of the C–C coupling reaction is illustrated.

Acid-catalyzed reaction of **3** with benzene in the presence of trifluoromethanesulfonic acid (TFSA) under reflux did not give hydroxybiphenyls (**4** and **5**) but phenol and aniline. Thus the amino group was modified by acylation to an acylamino group which would render to increase the leaving ability of the nitrogen moiety. N-Tosylation of 3 with *p*-tosyl chloride in pyridine gave *N*-tosyl-*O*-phenylhydroxylamine (**6**) as stable crystals. To a solution of **6** in benzene (50 equiv) was added a mixture of trifluoroacetic acid (TFA, 50 equiv) and TFSA (2.5 equiv), and the solution was allowed to stand at room temperature (or below) for 1 h. Products identified were 2-(**4**, 33%) and 4-hydroxybiphenyl (**5**, 16%), 4-trifluoromethanesulfonyloxyphenol (**7**, ~3%), a rearranged catechol derivative (**8**, trace), and *p*-toluenesulfonamide (85%) (Chart I). In the presence of only TFA (50 equiv), the reaction with benzene required the refluxing temperature, and the yields of **4** and **5** were 15 and 6%, respectively. Diphenyl ether and biphenyl were not detected in the reaction. Diphenyl ether was stable under the reaction conditions.

A similar reaction of the tosylate (**6**) in anisole gave a mixture of hydroxymethoxybiphenyls in 60% yield, which constituted of 2- (25%) and 4-hydroxy-2′-methoxybiphenyl (20%) and 2- (17%) and 4-hydroxy-4′-methoxybiphenyl (38%)

Chart I

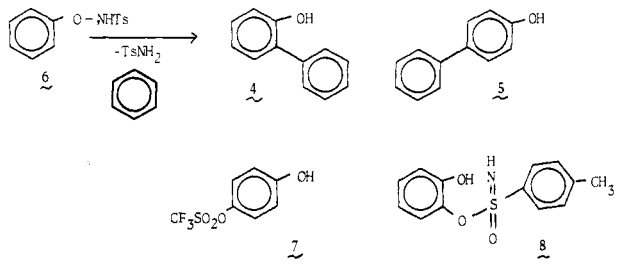


Chart II

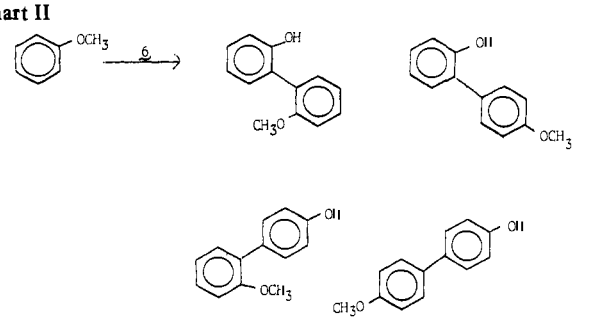
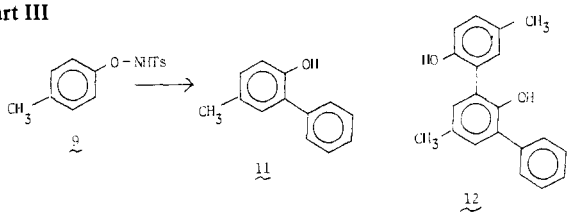


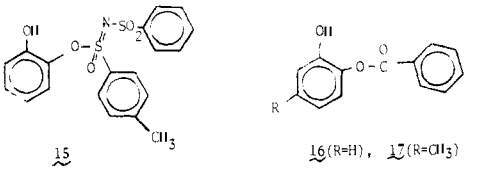
Chart III



(Chart II). Similarly reaction with phenol gave a mixture of dihydroxybiphenyls in 59% yield, which constituted of 2,2′- (27%), 2,4′- (22%), and 4,4′-dihydroxybiphenyl (51%). These reactions suggest that the reactive species from **6** is electrophilic. The *N*-tosylate (**9**) of *O*-(4-methylphenyl)hydroxylamine (**10**) is more reactive than **6**, probably because of the electron-releasing effect of the methyl group, and reacted with benzene at room temperature in the presence of TFA giving 2-hydroxy-5-methylbiphenyl (**11**, 26%). Reaction catalyzed by TFA (50 equiv)–TFSA (1 equiv) gave **11** (44%) and a secondary product (**12**, 9%) formed from **9** and **11** (Chart III). In this case, no phenylation at the para position occurred. This is a good contrast to the acid-catalyzed phenylation of *N*-(4-methylphenyl)hydroxylamine (**13**) with benzene where the major product was formed from the para attack.[1]

The *N*,*N*-ditosylamino group is expected to be a better leaving group. *N*,*N*-Ditosyl-*O*-phenylhydroxylamine (**14**) was prepared from **6** with tosyl chloride in the presence of NaH in THF. The reaction of **14** with benzene proceeded in the presence of TFA at 5 °C giving **4** (20%) and **5** (8%) and a rearranged compound (**15**, 30%).

The *N*-benzoate of **3** under similar conditions (TFSA, 50 equiv) gave **4** (15%), **5** (9%), and a rearranged compound (**16**, 40%). The *N*-benzoate of **10** also gave **11** (20%) and **17** (50%). In the absence of benzene, the yields of **16** and **17** were 78 and 77%, respectively. The formation of **8**, **15**, **16**, and **17** can be



interpreted by a closely related intramolecular mechanism of the rearrangement of *N*-phenyl-*N*-acylhydroxylamine[9] and the oxidation of phenol by benzoyl peroxide.[10]

The mechanism of the phenylation reaction is accounted for possibly by the heterolytic cleavage of the N–O bond to give PhO^+ from the following reasons: (1) the formation of **7** is well explained by the trapping of PhO^+ by trifluoromethanesulfonyloxy anion; (2) the modification of the nitrogen moiety as the better leaving groups and the substitution by methyl on the phenyl ring increase the reactivity of the N–O bond; (3) the electrophilic attack on phenol and anisole occurred; (4) the absence of biphenyl as a product as well as the lack of the effect of light, air, *m*-dinitrobenzene, and ascorbic acid[11] may eliminate a possible homolytic cleavage of N–O bond; and (5) the participation of a protonated phenoxenium ion[12] is less likely because the reaction could be catalyzed by the weaker acid TFA if the leaving ability of the nitrogen moiety is good and there is a different reactivity of **9** from **13**, in the reaction of **13** a protonated anilenium ion[1,4] having been suggested as the reactive species. Thus a possible pathway to **4** and **5** is shown in Scheme I, though we could not discuss whether PhO^+ is singlet or triplet. Another possible mechanism is a concerted one which is not ruled out by the present evidence.

The present reaction may be a good tool for a phenol–benzene coupling reaction which is frequently required for a natural product synthesis. An example of an intramolecular cyclization is shown in Chart IV. The reaction proceeded in tri-

Scheme I

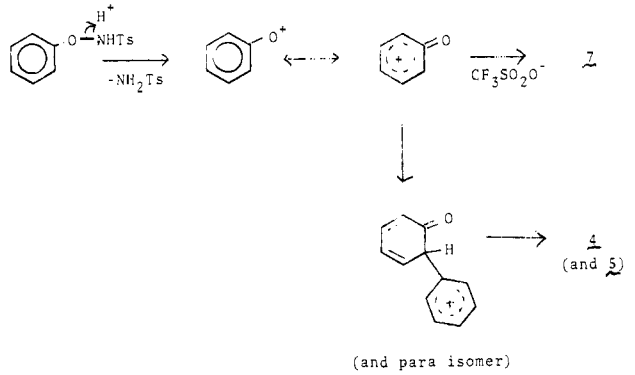
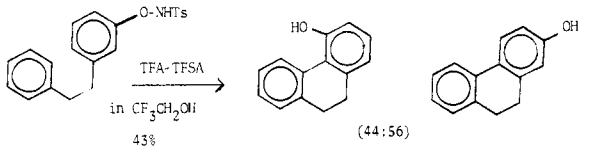


Chart IV



fluoroethanol in the presence of TFA (20 equiv)-TFSA (1 equiv).

References and Notes

(1) T. Okamoto, K. Shudo, and T. Ohta, *J. Am. Chem. Soc.*, **97**, 7184 (1975).
(2) E. Bamberger, *Justus Liebigs Ann. Chem.*, **424**, 233, 297 (1921). J. H. Parish and M. C. Whiting, *J. Chem. Soc.*, 4713 (1964).
(3) T. Ohta, K. Shudo, and T. Okamoto, *Tetrahedron Lett.*, 105 (1977).
(4) K. Shudo, T. Ohta, and T. Okamoto, *Tetrahedron Lett.*, 101 (1977).
(5) Arylhydroxamic acids: Y. Endo, T. Ohta, K. Shudo, and T. Okamoto, *Heterocycles*, in press.
(6) A. Ronlan and V. Parker, *J. Chem. Soc. C*, 3214 (1971); M. A. Schwarz, B. F. Rose, and B. Vishunuvajjala, *J. Am. Chem. Soc.*, **95**, 612 (1973); K. Dimroth, W. Umbach, and H. Thomas, *Chem. Ber.*, **100**, 132 (1967).
(7) R. A. Abramovitch, M. Inbasekaran, and S. Kato, *J. Am. Chem. Soc.*, **95**, 5428 (1973).
(8) R. A. Abramovitch, G. Alvernhe, and M. Inbasekaran, *Tetrahedron Lett.*, 1113 (1977).
(9) S. Oae and T. Sakurai, *Tetrahedron*, **32**, 2289 (1976), and references cited therein.
(10) D. B. Denney and D. Z. Denney, *J. Am. Chem. Soc.*, **82**, 1389 (1960).
(11) K. Shudo, and T. Okamoto, *Tetrahedron Lett.*, 1893 (1973).
(12) A. Nilsson, U. Palmquist, A. Ronlan, and V. Parker, *J. Am. Chem. Soc.*, **97**, 3540 (1975).

Yasuyuki Endo, Koichi Shudo, Toshihiko Okamoto*

Faculty of Pharmaceutical Sciences
University of Tokyo, Hongo, Tokyo, Japan

Received May 11, 1977

Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Iron(III) Porphyrin-Cyanide Complexes. Location of the Bound Cyanide Ion Resonance

Sir:

Cyanide ion is often chosen as an axial ligand for conversion of high-spin iron(III) porphyrins and hemoproteins to the low-spin form. Aqueous solution studies involving simple iron porphyrins indicate stepwise equilibria to form monocyano and dicyano species. Equilibrium quotients at 25 °C are on the order of 10^5 M^{-1}.[1] Very favorable cyanide ion binding has resulted in use of this ligand for recording nuclear magnetic resonance spectra of low-spin iron(III) porphyrins and hemoproteins.[2] ^1H NMR resonances in dicyano iron(III) porphyrin complexes show solvent dependence[3] and at low temperatures in methanol solution a specific aggregation process has been elucidated.[4] ^1H NMR spectra have been recorded for both monocyano and dicyano complexes in dimethyl sulfoxide solution and ligand exchange was not detectable on the NMR time scale at 65 °C.[5] ^{13}C NMR spectra of dicyano iron(III) porphyrin complexes have been reported, and, because a bound cyanide peak was not observed, it was initially concluded that rapid ligand exchange occurred.[6] During a more extensive ^{13}C NMR study we noted that the free cyanide ion peak was not shifted or broadened in presence of iron(III) porphyrins.[7] Such evidence is clearly indicative of slow cyanide exchange and subsequent use of carbon-13 enriched potassium cyanide has permitted observation of bound cyanide resonances. This report describes initial efforts directed at elucidation of ligand exchange kinetics, solution interactions, and spin delocalization mechanisms. Possible use of carbon-13 enriched cyanide ion as a probe for ferrihemoprotein structure is also under evaluation.

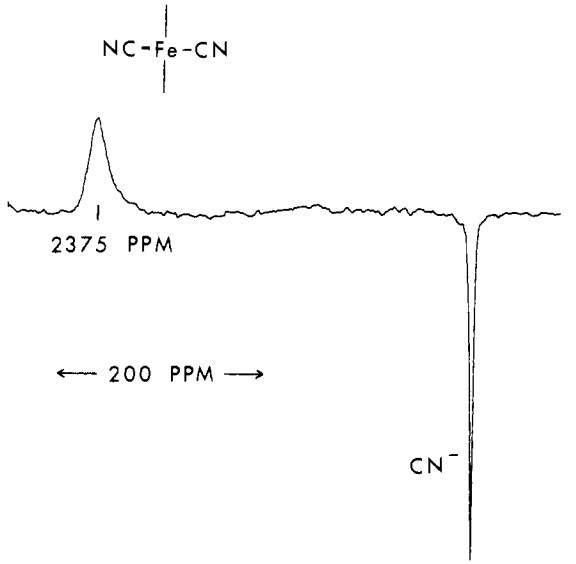


Figure 1. ^{13}C NMR spectrum of iron(III) deuteroporphyrin, 0.01 M, and 90% C-13 potassium cyanide, 0.05 M, in deuterium oxide at 26 °C. A 50-Hz line-broadening factor has been used.

Spectra were recorded at 22.6 MHz using a Bruker HX-90E pulsed Fourier transform spectrometer. Sweep widths of 20 KHz or 40 KHz were employed. From 20 000 to 500 000 transients were accumulated per spectrum at rates up to 15 pulses per second. Resonance positions are referenced to tetramethylsilane using dioxane as a secondary reference for deuterium oxide solutions. Upfield shifts are given positive values. Potassium cyanide enriched to 90 at. % carbon-13 was employed throughout the study. The ^{13}C resonance for potassium hexacyanoferrate(III) at natural abundance in aqueous solution was observed at +3583 ppm (26 °C) in close agreement with earlier broad-line NMR results.[8]

A typical upfield region ^{13}C NMR spectrum of a dicyano iron(III) porphyrin is shown in Figure 1. The resonance for excess free cyanide-hydrogen cyanide is "folded back" and inverted because the signal lies outside the spectral window. Excess potassium cyanide was employed to ensure complete ligation and to solubilize the iron(III) porphyrin via deprotonation of propionic acid groups. Dissolution with sodium deuterioxide and addition of potassium cyanide yielded equivalent results. The porphyrin resonances at natural abundance in carbon-13 are lost in the baseline. The broad, paramagnetically shifted signal corresponds to the bound cyanide species. A potassium cyanide titration of hemin *c* dissolved in pD 12.5, 0.1 M phosphate buffer revealed that the observed signal represents the dicyano complex. A resonance has not yet been located for the monocyano complex, perhaps because of exchange broadening. Line widths for the bound resonances in dicyano complexes are in the range of 360 ± 50 Hz with the exception of the iron(III) 2,4-dibromodeutero-