be crystallized but was essentially one component by tlc analysis: nmr ($CDCl_3$) δ 0.73 (s, 3, 18 H), 0.92 (s, 3, 19 H), 1.38 (d, 3, J = 7 Hz, 21 H), 2.01, 2.03, 2.04, and 2.13 (4 s, 6, $OC(=O)CH_3$ and $NC(=O)CH_3$), 2.29 (s, 6, $N(CH_3)_2$), 2.58–3.88 (m, 5, 3 H, 16 H, 20 H, NCH_2), 4.18 (m, 2, OCH_2), 4.90 (m, 1, 6 H), and 7.14–7.68 ppm (m, 15, aromatic H).

Treatment of the acetate **5b** (0.295 g) in acetic acid (25 ml) for 7.5 hr at 56° and then pouring the mixture into ice-water afforded a precipitate which was collected. This was triphenylcarbinol (0.05 g). The filtrate was made basic with 30% potassium hydroxide. The resultant precipitate (0.156 g) was collected and put on two preparative thin layer chromatography plates (200 × 200 × 1 mm) (silica gel G) and developed in the system 85% of benzene–acetone–water (2:1:2) (upper phase) and 15% of methanol. The less polar band (0.127 g) (*ca.* 9–9.5 cm from the origin) was collected and crystallized from acetone–hexane to give **5c** (0.030 g): mp 203–203.5° dec; $[\alpha]^{25}D$ −9.6° ($CHCl_3$); ir 3410, 1750, 1642, 1630, and 1230 cm^{-1}; nmr ($CDCl_3$) δ 0.78, 0.79 (d, 3, 18 H), 0.99 (s, 3, 19 H), 1.41 (d, 3, J = 6 Hz, 21 H), 2.03, 2.04 (d, 3, $NC(=O)CH_3$), 2.11, 2.17 (d, 3, $OC(=O)CH_3$), 2.31 (s, 6, $N(CH_3)_2$), 3.05–3.90 (m, 5, 3 H, 16 H, 20 H, NCH_2), 4.18 (m, 2, OCH_2), and 5.34 ppm (m, 1, 6 H); nmr at 90° ($CDCl_3$ + CD_3OD) δ 0.80 (s, 3, 18 H), 1.00 (s, 3, 19 H), 1.41 (d, 3, J = 7 Hz, 21 H), 2.03 (s, 3, $NC(=O)CH_3$), 2.12 (s, 3, $OC(=O)CH_3$), and 2.32 ppm (s, 6, $N(CH_3)_2$); mass spectrum (70 eV) m/e 488, 316, 172.

Anal. Calcd for $C_{29}H_{48}N_2O_4$ (488.69): C, 71.27; H, 9.90; N, 5.73. Found: C, 71.54; H, 9.86; N, 5.72.

The more polar band from the preparative plate (6.0 mm from the origin) gave a crude compound (0.03 g) which had physical measurement suggesting **5d** as its structure: nmr (DMSO-d_6) δ 0.68, 0.70 (d, 3, 18 H), 0.92 (s, 3, 19 H), 1.98 2.01 (d, 3, $NC(=O)CH_3$), 2.22 (s, 6, $N(CH_3)_2$), 4.50 (d, 1, CHOH), 4.75 (m, 1, CH_2OH), and 5.28 ppm (m, 1, 6 H); mass spectrum (70 eV) m/e 446, 316, 130.

16β-Dimethylaminopregn-5-en-3β-yl Trityl Ether (6). A.—A mixture of the tosylate **3a** (0.5 g) and lithium aluminum hydride (1.0 g) in tetrahydrofuran (250 ml) (the steroid was not in solution) was stirred at room temperature for 15 min and then stirred and refluxed for 5 hr. The resultant mixture was worked up as in the preparation of **2b**. Removal of the solvent *in vacuo* afforded a glass which was crystallized from acetone–methanol to give **6** (0.26 g): mp 159–160° (recrystallization did not change the melting point); $[\alpha]^{25}D$ −27°; ir 765, 760, 747, 705, and 696 cm^{-1}; nmr ($CDCl_3$) δ 0.68 (s, 3, 18 H), 0.96 (s, 3, 19 H), 2.23 (s, 6, $N(CH_3)_2$), 4.91 (m, 1, 6 H), and 7.16–7.67 ppm (m, 15, aromatic H).

Anal. Calcd for $C_{42}H_{53}NO$ (587.85): C, 85.81; H, 9.09; N, 2.38. Found: C, 86.17; H, 9.25; N, 2.17.

B.—A mixture of the diene **4a** (0.29 g) and 10% palladium on charcoal (0.03 g) in tetrahydrofuran (20 ml) was stirred and treated with hydrogen at room temperature and atmospheric pressure for 1 hr when approximately 1 mol equiv of hydrogen was absorbed. After filtration of the catalyst, the tetrahydrofuran was removed from the filtrate *in vacuo* to give an amorphous solid. Crystallization from acetone–methanol afforded **6** (0.25 g), mp 160–161°. The infrared spectrum was identical with that of the sample prepared in A.

Registry No.—**1b**, 28463-69-6; **2b**, 28463-70-9; **3a**, 28463-71-0; **3b**, 28463-72-1; **4a**, 28463-73-2; **4b**, 28463-74-3; **5a**, 28463-75-4; **5b**, 28463-76-5; **5c**, 28463-77-6; **6**, 28463-78-7.

The Reduction of Aromatic Nitro and Related Compounds by Dihydroflavins

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Received October 22, 1970

The reduction of nitrobenzene by dihydroflavins (or dihydroisoalloxazines) in organic solvents leads to *N*-phenylhydroxylamine and flavins (or isoalloxazines). Nitrosobenzene is very rapidly reduced to *N*-phenylhydroxylamine, and azobenzene leads to hydrazobenzene. Azoxybenzene is sluggishly reduced to hydrazobenzene and aniline. *N*-phenylhydroxylamine also slowly oxidizes reduced flavins, likely *via* disproportionation (to nitrosobenzene and aniline) followed by reaction of the product with dihydroflavin. The reactions of nitrobenzene and six para-substituted nitrobenzenes with dihydro-3-methyllumiflavin in DMF over a range of concentrations follow good second-order kinetics (first order in each reactant). The second-order rate constants fit a Hammett relationship using σ^- substituent constants, ρ^- = +3.6. On the basis of these data along with their relationship to electrochemical and other aromatic nitro reduction methods, a tentative initial step involving electron transfer is proposed. The azobenzene reaction also displays first-order behavior in each reactant (second order overall). No intermediates were observed spectrophotometrically in any of these systems. Aliphatic nitro compounds are unreactive to dihydroflavins.

As part of our studies of the redox chemistry of flavins with organic molecules related to substrates for flavoenzymes,[2] we have investigated reactions between oxidized and reduced flavins (see Scheme I) and the redox states between (and including) nitrobenzene and aniline. The flavoenzymes involved in nitrate reduction and in various metabolic pathways may perform reactions related to those described in this paper.[3] None of the compounds reported in this study reduced flavin, but as reported below several of the oxidation states of nitrobenzene oxidized reduced flavins. Aliphatic nitro compounds were unreactive.

Results

Nitrobenzene and Substituted Nitrobenzenes.—Aromatic, but not aliphatic, nitro compounds oxidize reduced flavins to the normal oxidized flavins in organic solution (isolated chromatographically and identified by thin layer chromatography and spectrally). In the case of nitrobenzene itself the reaction is rather sluggish, requiring approximately 2 days for complete reoxidation of 10^{-4} M dihydroflavin with 10^{-2} M nitrobenzene in dimethylformamide (DMF), dimethyl sulfoxide (DMSO), or acetonitrile.

Thin layer chromatography of the reaction mixture showed major spots for *N*-phenylhydroxylamine and aniline plus unreacted starting material. Every work-up procedure that we have used in preparative experiments has, however, led to destruction of the phenylhydroxylamine with production of aniline. There is evidence as well that phenylhydroxylamine is reduced (by a circuitous route discussed below) to aniline by dihydroflavin. Ultimately in the nitrobenzene reaction

(1) NSF Undergraduate Research Participant, Summer 1969.

(2) M. J. Gibian and D. V. Winkelman, *Tetrahedron Lett.*, **44**, 3901 (1969).

(3) A leading reference is K. Yagi, Ed., "Flavins and Flavoproteins," University Park Press, Baltimore, Md., 1968.

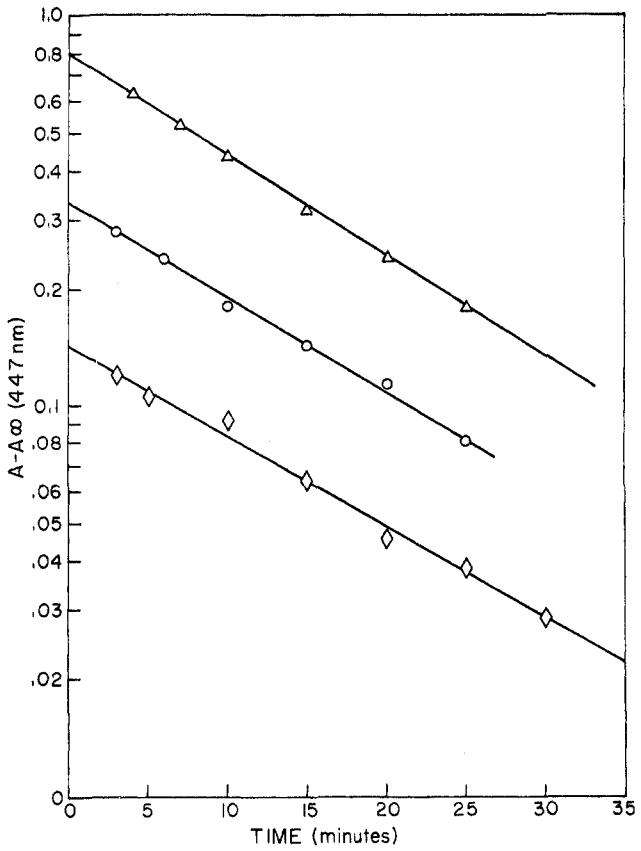
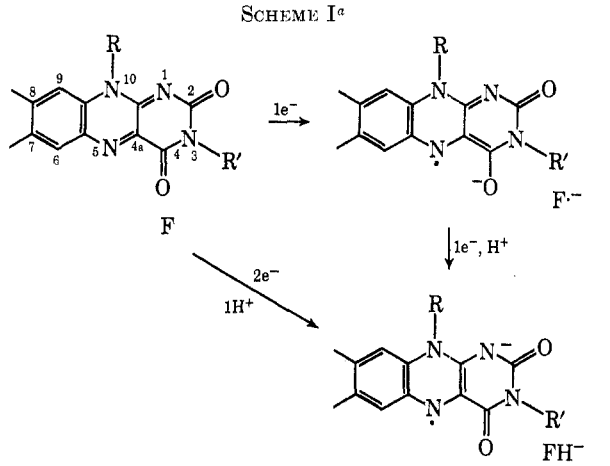


Figure 1.—Representative pseudo-first-order plots of the appearance of oxidized 3-methyllumiflavin (at 447 nm) with time in the presence of 4×10^{-3} *M* *p*-chloronitrobenzene in DMF: △, 1.0×10^{-4} *M* FH_2; ○, 5.0×10^{-5} *M* FH_2; ◇, 2.5×10^{-5} *M* FH_2.

SCHEME I[a]



[a] Only one tautomeric or resonance form for each state has been drawn. Flavins are 7,8-dimethylisoalloxazines.

mixture, a 59% yield of aniline is obtained as determined by gas chromatography (based on three successive 2-electron reductions; *i.e.*, a 3 to 1 stoichiometry of nitrobenzene to flavin).

The kinetics of the oxidation of dihydroflavin by nitrobenzene and by substituted nitrobenzenes were studied in DMF. Electron-withdrawing groups on the nitrobenzene ring significantly accelerate the rate of flavin oxidation. For reasons of experimental facility, the thorough kinetics of oxidation of dihydroflavins by *p*-chloronitrobenzene was studied. The concentration of 3-methyllumiflavin was varied from 2.5×10^{-5} *M* to 1.0×10^{-4} *M*, and that of *p*-chloronitrobenzene was varied from 2×10^{-3} *M* to 8×10^{-3} *M*. The reactions were run under argon in Schlenk tubes which were spectrophotometer cells at the bottom. A typical run was pseudo zero order in the nitro compound and first order in flavin (Figure 1). Variation of the concentration of each reactant showed the reaction to be second order overall (typical plot shown in Figure 2) over the range of our experiments. Table I gives the second-order rate constants for a series

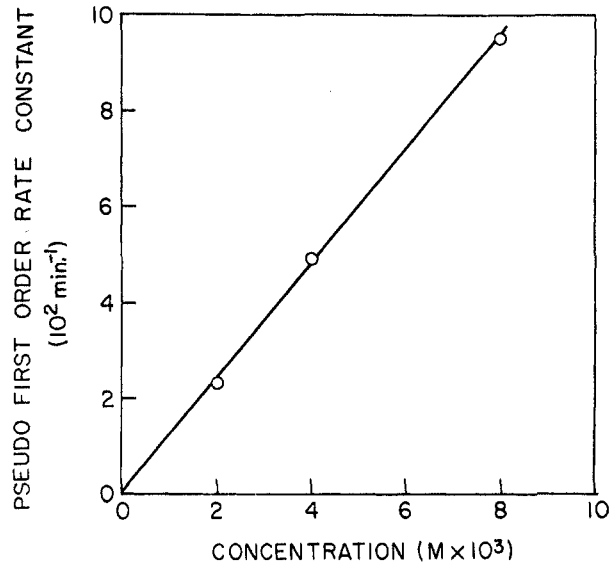


Figure 2.—Plot of the pseudo-first-order rate constants from the data in Figure 1 (from variation of initial dihydro-3-methyllumiflavin) *vs.* concentration of *p*-chloronitrobenzene. Each point represents at least duplicate runs of each of three different FH_2 concentrations. The slope is the second-order rate constant given in Table I.

TABLE I
SECOND-ORDER RATE CONSTANT FOR OXIDATION OF DIHYDRO-3-METHYLLUMIFLAVIN BY SUBSTITUTED NITROBENZENES

X (X–C₆H₄–NO₂)	Registry no.	k_2 (M^{-1} min^{-1})
–OMe	100-17-4	($\sim 3.3 \times 10^{-2}$)
$-CH_3$	99-99-0	1.3×10^{-1}
–H	98-95-3	3.3×10^{-1}
–Cl	100-00-5	$1.3 \times 10^{+1}$
$-\overset{O}{\overset{\|}{C}}CH_3$	100-19-6	$6.3 \times 10^{+2}$
–CN	619-72-7	$2.9 \times 10^{+3}$
$-\overset{O}{\overset{\|}{C}}H$	555-16-8	$4.4 \times 10^{+3}$

of substituted nitrobenzenes under the same conditions. Data were obtained by varying the concentration of each nitro compound over at least severalfold, good second-order kinetics being obtained in each case. Figure 3 is a Hammett plot for this reaction using σ^- parameters. The fit using normal σ substituent constants is quite poor for the *p*-cyano and *p*-formyl groups, predicting the wrong order of reactivity for these and the *p*-acetyl groups by large amounts.

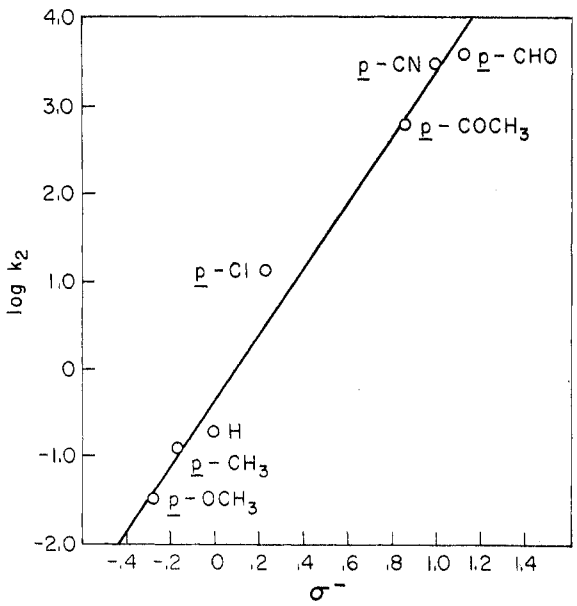


Figure 3.—Linear-free-energy correlation of the second-order rate constants in Table I (M^{-1} min^{-1}) with Hammett σ^- substituent constants.

Least-squares analysis gives $\rho^- = +3.62 \pm 0.23$ (intercept $= 0.28 \pm 0.15$), $r = 0.99$.

Nitrosobenzene.—Reduced flavins are oxidized by nitrosobenzene as fast as can be visually observed upon mixing. Normal oxidized flavin can be recovered quantitatively, and the substrate product is *N*-phenylhydroxylamine, as shown by recovery of azoxybenzene when a twofold excess of nitrosobenzene is added to flavin.[4,5] Reactions 1 and 2 describe the chemistry

$$C_6H_5NO + FH_2 \longrightarrow C_6H_5NHOH + F \quad (1)$$

$$C_6H_5NHOH + C_6H_5NO \longrightarrow C_6H_5N{=}NC_6H_5 + H_2O \quad (2)$$
$$\downarrow$$
$$O$$

involved here. We actually isolate a 39% yield of azoxybenzene from the reaction mixture. Thin layer chromatography shows that it is the only product, and we feel that the yield of isolated product is not higher because of the isolation procedure.

***N*-Phenylhydroxylamine.**—Dihydroflavins are very slowly oxidized to flavins by *N*-phenylhydroxylamine. After a significant number of days we find mostly aniline along with various condensation products (azoxy and azobenzenes) in the reaction mixture. It is known that disproportionation between two molecules of *N*-phenylhydroxylamine leads to aniline and nitrosobenzene (eq 3) and that subsequent condensation as

$$2C_6H_5NHOH \longrightarrow C_6H_5NO + C_6H_5NH_2 \quad (3)$$

in eq 2 leads to azoxybenzene. Both nitrosobenzene and azoxybenzene are capable of oxidizing reduced flavin. Furthermore, nitrosobenzene would lead to (a) phenylhydroxylamine on reduction (eq 1) which then recycles or (b) to azoxybenzene (eq 2) which is slowly reduced, so that ultimately the products should be aniline and those resulting from azoxybenzene reduction (*vide infra*).

(4) (a) S. Oae, T. Fukumoto, and M. Yamagami, *Bull. Chem. Soc. Jap.*, **36**, 728 (1963); (b) G. A. Russell, E. J. Geels, F. J. Smentowski, K.-Y. Chang, J. Reynolds, and G. Kaupp, *J. Amer. Chem. Soc.*, **89**, 3821 (1967).

(5) I. T. Millar and H. D. Springall, "Sidgwick's Organic Chemistry of Nitrogen," 3rd ed, Clarendon Press, Oxford, England, 1966, p 306.

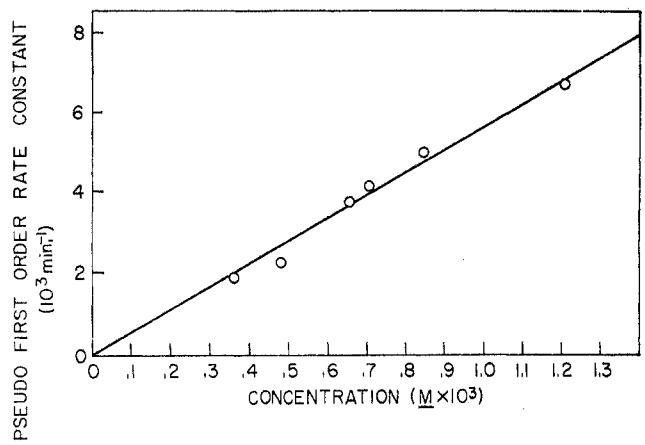


Figure 4.—Plot of the pseudo-first-order rate constants for the reduction of azobenzene obtained by varying dihydro-3-methyllumiflavin concentration (from 4×10^{-5} *M* to 1.3×10^{-4} *M*) *vs.* azobenzene concentration. The slope is the second-order rate constant (see text).

Azobenzene.—Azobenzene is an efficient oxidizing agent for dihydroflavin, producing a 62% isolated yield of hydrazobenzene along with a quantitative yield of oxidized flavin (eq 4). This reaction is second-

$$C_6H_5N{=}NC_6H_5 + FH_2 \longrightarrow C_6H_5NHNHC_6H_5 + F \quad (4)$$

order overall, first order in reduced flavin and first order in azobenzene. Figure 4 is a plot of the pseudo-first-order rate constant for dihydroflavin oxidation *vs.* initial azobenzene concentration. The slope, which is the second-order rate constant for the reaction, is 6.1 ± 0.4 M^{-1} min^{-1} (correlation coefficient 0.99). The rate is thus similar to that of the nitrobenzene reaction. Examination of spectra *vs.* time showed no buildup of intermediates, and hydrazobenzene was stable to both oxidized and reduced flavin.

Azoxybenzene.—Dihydroflavin and azoxybenzene slowly produce oxidized flavin (100%) along with aniline (~33%) and hydrazobenzene (~67%). This reaction is quite slow compared to the azobenzene and nitrobenzene reactions but of about the same rate as the reaction of *N*-phenylhydroxylamine.

Discussion

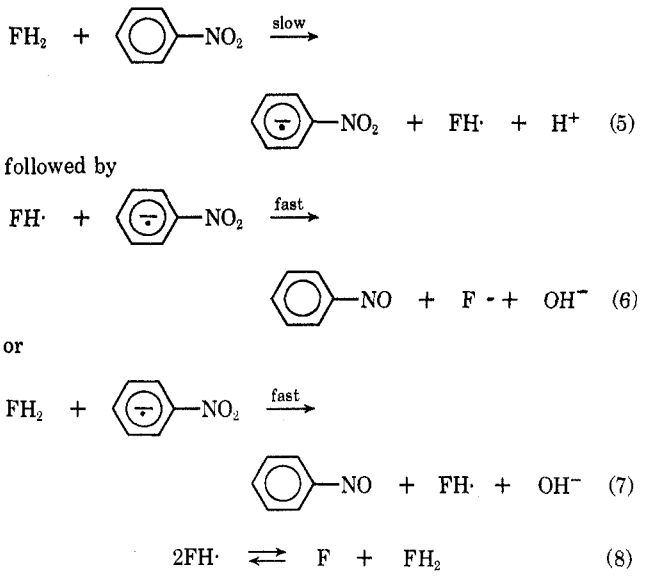
The patterns for reduction of nitrobenzene and its partially reduced derivatives by dihydroflavin follow a pattern not dissimilar from that of other organic reducing agents. The mechanisms of reduction of these functional groups have not been studied in detail for many of the normal reducing agents, but the general observations in this study would seem to place dihydroflavin at approximately the strength and specificity of metallic zinc in neutral aqueous solution. Lithium aluminum hydride sluggishly reduces most of these derivatives, but most of the other metal hydrides are inert (except that all reducing agents essentially are rapid and efficient with nitroso compounds).[6] Catalytic hydrogenation, on the other hand, is quite efficient in the reduction of all the oxidation states. Dihydroflavin is thus placed between $LiAlH_4$ and catalytic

(6) J. March, "Advanced Organic Chemistry," McGraw-Hill, New York, N. Y., 1968, p 890 ff.

hydrogenation in specificity and similar to the dehydropyridines.[7]

We tend to favor, from several lines of reasoning, a pathway involving successive one-electron transfers as the mode of reduction of the nitro compounds by dihydroflavins. Although *p*-nitrobenzaldehyde is very rapidly reduced by dihydroflavin, only the nitro group reacts. With metal hydrides (direct hydride donors), on the other hand, aldehyde functionalities are *more* readily reduced than are nitro compounds.[6] Radical anions of nitroaromatic compounds are highly stabilized, and evidence for their intermediacy in the reduction of nitrobenzenes by sodium arsenite, sodium ethoxide, or glucose is the observation of the transient epr spectrum of the nitrobenzene radical ion, the nitrosobenzene radical ion, and ^{18}O and ^{15}N scrambling.[4a] Scheme II depicts a pathway involving a slow one-

SCHEME II



electron transfer step (eq 5) followed by reaction 6 or by reactions 7 and 8 to arrive at nitrosobenzene for the nitrobenzene reaction. Rapid reduction by eq 1 would follow.

The necessity of using σ^- substituent parameters in our Hammett plot indicates that negative charge is directly conjugated with substituents in the transition state. This could, of course, be either a two- or one-electron transfer, but the following consideration may shed some light on this question. The substituent effect on the half-wave reduction potential of substituted nitrobenzenes in DMF has been investigated,[8] it having been found that ρ_π (volts) is equal to approximately +0.35 to +0.40 V. The σ^- parameters gave a better fit of the data. Converting ρ_π(v) into the same units as that obtained in normal mechanistic organic chemistry[9] $[(\alpha n_a F/RT)\rho_\pi = \rho]$, ρ^- for the electrochemical reduction is equal to +3.0 to +3.4. In the present study, ρ^- was +3.6. It is highly probably that the electrochemical reduction is in fact a one-electron process as the rate-determining step.[9b] The implication is that, in the transition state for the flavin reduction and in the electrochemical process, roughly the same negative charge is transferred onto a nitrobenzene nucleus. Russell, *et al.*,[10] have studied the substituent effects on the one-electron transfer from fluorenide anion and from acetophenone enolate to substituted nitroaromatics. Solvents were different from those used in our study, but the two ρ values were approximately 2.0 and 2.7, respectively.

The literature contains numerous references to a value of σ for the *p*-formyl group of +0.21, which was found to be in error because of an unrecognized chemical reaction taking place in the original study. By ionization of terephthalaldehyde, Humffray, *et al.*,[11] report a more reasonable value of +0.45. In the course of our studies we had to choose a value for this group (ultimately, the σ^- parameters were found to give the best fit) and calculated it from Taft's nmr data, using $\sigma^0 = \sigma_0^R + \sigma_I$.[12] The value was +0.55. Thus the value of approximately +0.5 is clearly preferred from two independent studies and is more in accord with that expected, based on the acetyl (σ_p = +0.52) and the carbethoxyl groups (σ_p = +0.52).[13]

Experimental Section

Reagents.—Solvents used in product studies were reagent grade materials, distilled before use for kinetic studies. Riboflavin (Aldrich) was recrystallized from 2 *M* acetic acid. Lumiflavin,[14] 3-methyllumiflavin,[15] 3-benzyllumiflavin (*via* 3-benzylbarbituric acid),[16] 10-phenylisoalloxazine,[17] and 3-benzyl-10-phenylisoalloxazine (*via* benzyl bromide and 10-phenylisoalloxazine analogously to the flavin series[15]) were synthesized by published routes. Nitrobenzene (MCB) was distilled before use. Substituted nitrobenzenes (MCB reagents), azoxybenzene (Eastman), azobenzene (MCB), hydrazobenzene (Aldrich), and nitrosobenzene (Aldrich) were recrystallized from ether–pentane. All gave satisfactory melting points. *N*-Phenylhydroxylamine was synthesized by a standard procedure, mp 80–82° (lit. 81°).[18]

Exploratory and Kinetic Experiments.—All reactions were run in the rigorous absence of oxygen. This was accomplished by bubbling argon through all solvents and reagent solutions for at least 0.5 hr before initiating reactions. Care was taken to rigorously exclude light from all stock solutions and reactions except while spectra were being recorded.

Reactions were run in several types of vessels, but all of these were modified to effectively be Schlenk tubes. In many of these experiments, the tubes were constructed from 1.5 in. of 1-cm square Pyrex tubing rounded off and sealed at the bottom and fused at the top onto ordinary cylindrical Pyrex tubing terminating in an ⅋ 14/20 or 19/22 inner joint. Just below the joint was a side arm with a stopcock at approximately 45° from the vertical. This sidearm was used for blowing argon over the top of the solution while adding reagents. To cover the tube an outer ⅋ joint of the same bore terminating in a stopcock was used. In this manner, solutions could be deoxygenated, reagents added rapidly, and spectra recorded very shortly after mixing. Additional reagents could be injected at any time without admit-

(7) D. C. Dittmer and J. M. Kolyer, *J. Org. Chem.*, **27**, 56 (1962).

(8) (a) P. Zuman, "The Elucidation of Organic Electrode Processes," Academic Press, New York, N. Y., 1969, p 132; (b) A. H. Maki and D. H. Geske, *J. Amer. Chem. Soc.*, **83**, 1852 (1961).

(9) (a) C. L. Perrin, *Progr. Phys. Org. Chem.*, **3**, 292 (1965). (b) We have assumed, arbitrarily, that αn_a = 0.5. This would seem not unreasonable for this chemical situation, since α is generally between 0.3 and 0.7, and n_a in a kinetic sense may realistically be assumed to be close to 1.0 electron.

(10) G. A. Russell, *et al.*, *Advan. Chem. Ser.*, **51**, 1112 (1965).

(11) A. A. Humffray, J. J. Ryan, J. P. Warren, and Y. H. Yung, *Chem. Commun.*, **23**, 610 (1965).

(12) R. W. Taft and I. C. Lewis, *J. Amer. Chem. Soc.*, **81**, 5343 (1959).

(13) C. D. Ritchie and W. F. Sager, *Progr. Phys. Org. Chem.*, **2**, 334, (1964).

(14) P. Hemmerich, S. Fallab, and H. Erlenmeyer, *Helv. Chim. Acta*, **39**, 1242 (1956).

(15) P. Hemmerich, *ibid.*, **47**, 464 (1964).

(16) J. B. Dickey and A. R. Gray, "Organic Syntheses," Collect. Vol. II, Wiley, New York, N. Y., 1943, p 60.

(17) R. Kuhn and F. Weygand, *Ber.*, **68**, 1282 (1935).

(18) O. Kamm, "Organic Syntheses," Collect. Vol. I, Wiley, New York, N. Y., 1941, p 445.

ting air by reattaching the vessel to the manifold, keeping a stream of argon blowing, and adding deoxygenated reagents *via* a gas-tight syringe. Flavins at the reduced level could be retained in the vessels for quite extended periods without any reoxidation, and many reopenings and closings of the tubes could be performed without the introduction of air.

Flavins were reduced by the addition of very small aliquots of freshly prepared sodium dithionite ($Na_2S_2O_4$) solution in 10^{-3} *M* NaOH or by catalytic hydrogenation over Pd on activated silica gel (followed by anaerobic filtration of reduced flavin). The dithionite solutions were standardized by titration of oxidized flavin in separate experiments and were stable in dilute base for several hours. Catalytic hydrogenation was used primarily for product isolation experiments, but it was shown that spectral observations were identical with those in dithionite-reduced flavin experiments.

Kinetic runs were followed in the visible region on a Cary 14 spectrophotometer by observing the increase in flavin absorbancy upon oxidation. For slow reactions the spectrum was scanned at appropriate time intervals while faster runs were continuously monitored at the λ_{max} for oxidized material (447 nm for flavins, 437 nm for isoalloxazines).

Product Isolations. Nitrobenzene.—A DMF solution (100 ml) of 3-methyllumiflavin (0.138 g, 0.511 mmol) in a 250-cc round-bottom flask equipped with a Schlenk adapter at top was thoroughly deoxygenated by argon bubbling and reduced with 1.0 equiv of dithionite solution. Nitrobenzene (3.0 g, 24 mmol) was added anaerobically and the solution allowed to stand under a positive pressure of argon in the dark for 1 day. Upon opening, tlc showed major spots for nitrobenzene, *N*-phenylhydroxylamine, aniline, and a very slight amount of azoxybenzene. The bulk of the solution was passed through a silica gel column using ether–pentane (3:1) as eluent to remove the flavin (which is strongly retarded). Tlc of the chromatographed solution still contained *N*-phenylhydroxylamine and aniline. The solution was stripped down and then vacuum distilled. At this point, large amounts of aniline were observed on tlc (see *N*-phenylhydroxylamine below).

10-Phenylisoalloxazine (0.870 g, 3.00 mmol) in 1.5 l. of methanol was deoxygenated and reduced as above, 6.0 g (48 mmol) of nitrobenzene was added, and the reaction was allowed to proceed for 3 days. Methanol was removed at room temperature and the residue passed through silica gel to remove isoalloxazine. The total product mixture was then taken down to 45.0 ml and analyzed by glpc. Comparison to an external standard showed there to be a total of 0.055 g (0.59 mmol) of aniline. Based on net six-electron reduction, the theoretical yield of aniline (based on flavin) is 0.093 g (1.0 mmol); thus the yield represents 60% of the reducing equivalents from reduced flavin.

Nitrosobenzene.—Preliminary experiments with equimolar dihydroflavin and nitrosobenzene showed aniline and hydrazobenzene to be present. The reaction is complete almost immediately. In two careful experiments, 0.270 g (1.0 mmol) of 3-methyllumiflavin in 110 ml of DMF was deoxygenated and reduced as usual. Analysis on the Cary 14 showed that the flavin was 66% reduced (0.66 mmol). To this solution 0.21 g (2.02 mmol) of nitrosobenzene was added. Tlc showed only azoxybenzene as the product before and after removal of the DMF by vacuum distillation. Extraction with pentane and crystallization yielded 0.050 g (0.25 mmol) of azoxybenzene of high purity, a 38% overall yield.

***N*-Phenylhydroxylamine.**—*N*-Phenylhydroxylamine, allowed to stand in either methanol or DMF in the presence or absence of flavin or dihydroflavin, produced significant amounts of aniline in a few days. Upon removal of solvents from these solutions, significant quantities of tarry material were obtained. It was concluded that further attempts at product isolation would not be worthwhile.

Azobenzene.—3-Methyllumiflavin (0.0880 g, 0.32 mmol) in 100 ml of DMF was deoxygenated and reduced as usual with dithionite. Azobenzene (0.338 g, 1.86 mmol) was added and the solution allowed to stand for 3 days. Tlc showed that both hydrazobenzene and azobenzene were present in the reaction mixture. After passing the reaction mixture through a silica gel column to remove flavin and rechromatographing the eluate, the isolated yield of hydrazobenzene was 0.037 g (0.20 mmol), 62% overall based on dihydroflavin.

Azoxybenzene.—3-Methyllumiflavin (0.0071 g, 0.026 mmol) in 250 ml of methanol was deoxygenated and reduced as usual, 0.047 g (0.24 mmol) of azoxybenzene was added, and the solution was allowed to stand for 3 days. At the end of this time gas chromatography showed the presence of azoxybenzene, hydrazobenzene, and aniline. The ratio of hydrazobenzene to aniline was about 2 to 1. Flavin was removed from the solution by the usual method, and then the entire product mixture was taken down to 5.0 ml for glpc analysis. Based on an external standard added to this solution, the total yield of hydrazobenzene and aniline is close to 100% with a ratio of hydrazobenzene to aniline of 2 to 1.

Registry No.—Dihydro-3-methyllumiflavin, 23542-57-6; nitrosobenzene, 586-96-9; azobenzene, 103-33-3; azoxybenzene, 495-48-7.

Acknowledgments.—The authors thank the National Institutes of Health (GM-15100) and the Research Corporation for financial support of this work. We are also grateful to Mr. James Rynd, who made some of the initial observations upon which this paper is based.