2,5-Diamino-*p*-benzoquinone Derivatives as Photosystem I Electron Acceptors: Synthesis and Electrochemical and Physicochemical Properties

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A series of 2,5-diamino-*p*-benzoquinone derivatives have been prepared and their physicochemical properties studied. The sensitivity of their photoreduction potential to 3-(3,4-dichlorophenyl)-1,1-dimethylurea, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone, and KCN, as well as the photosystem I (PSI) activity, suggests that the reduction of 2,5-diamino-*p*-benzoquinone derivatives in the illuminated thylakoid is at the primary electron acceptor of PSI and it is reversible. The half-wave potentials of these compounds to their corresponding radical anions in an aprotic medium such as acetonitrile were found to be comparable with the midpoint potential values of the electron transport carriers at the reducing site of PSI. The strong reductant produced by PSI is really more accessible to the strong lipophilic electron acceptor. These lipophilic *p*-benzoquinone derivatives can reach the carriers inside the thylakoid membrane more easily than the ionic electron acceptor. The accepting electron properties of these compounds at PSI are similar to those of the bipyridinium herbicides.

Keywords: *2,5-Diamino-p-benzoquinone derivatives; photosystem I; electron acceptors; cyclic voltammetry; electrochemical reduction; half-wave potentials*

INTRODUCTION

Quinone redox systems play an important role as electron and proton carriers in the photosynthetic electron transport system of photosynthetic bacteria, algae, and higher plants, as well as in mitochondria. The electron transport chain of chloroplasts contains Q_A and Q_B as the quinone cofactors in the reducing side of photosystem II, bound by D_2 and D_1 proteins (Arntzen and Pakrasi, 1986, and references cited therein), respectively. On the other hand, PQH_2 cofactors of the cytochrome b_6f complex are bound to b_6 and/or the Rieske iron sulfur protein (Trebst, 1985, and references cited therein), and phylloquinone cofactor is part of the core complex of PSI at the A_1 site (Itoh and Iwaki, 1989). In many cases, quinone derivatives (and/or analogues) are used as electron donors, acceptors, redox mediators, or inhibitors of natural reactions in the bioenergetics field.

Urea and phenol type PSII herbicides are known to inhibit photosynthetic electron transport at the reducing site of PSII, at the level of the secondary two-electron accepting plastoquinone Q_B. However, the herbicidal action of some quinones is similar to that exhibited by the bipyridinium herbicides (i.e. paraquat and diquat) (Baker and Percival, 1990, and references cited therein) due to their capacity to intercept electrons by competing with the natural substrate ferredoxin. Furthermore, hydrophobicity of quinones is important for binding and inhibition or diverting electrons away from the A_1 site (Itoh and Iwaki, 1989) and the bacterial Q_A site (Warncke et al., 1987). Moreover, synthetic quinones with lower midpoint potential values between those of A_o and the iron−sulfur center Fx inhibit electron flow at the A_1 level, in A_1-depleted PSI particles (Itoh and Iwaki, 1989).

While searching for chemicals with potential herbicide activity that affect the reducing site of PSII, we have discovered a group of compounds, referred to as 2,5-diamino-*p*-benzoquinone derivatives (compounds **2**−**7**) (Figure 1), which behave as electron acceptors at the reducing site of PSI. Their analytical and spectroscopic characterizations, as well as their redox potential determination in aprotic medium, partition coefficients reported as log *P* values (Fujita et al., 1964), and extinction coefficients, have been investigated. The study of the redox behavior of these compounds using cyclic voltammetry has made possible the determination of the one-electron reduction potential for the couple $Q/Q^{\bullet-}$ in a nonaqueous environment. The half-wave potential for the reduction of this class of compounds to the corresponding anion radical permitted us to locate the site of electron acceptance at PSI.

MATERIALS AND METHODS

Chemicals and Solvents. All reagents used were commercially available. *p*-Benzoquinone (compound **1**), benzylamine, *o*-aminophenol, (±)-α-methylbenzylamine, (+)-α-methylbenzylamine, (−)-norpseudoephedrine, and (+)-norephedrine were from Aldrich. Tricine, MV, sorbitol, sucrose, ADP, DCPIP, DBMIB, DCMU, and diphenyl carbazide were from

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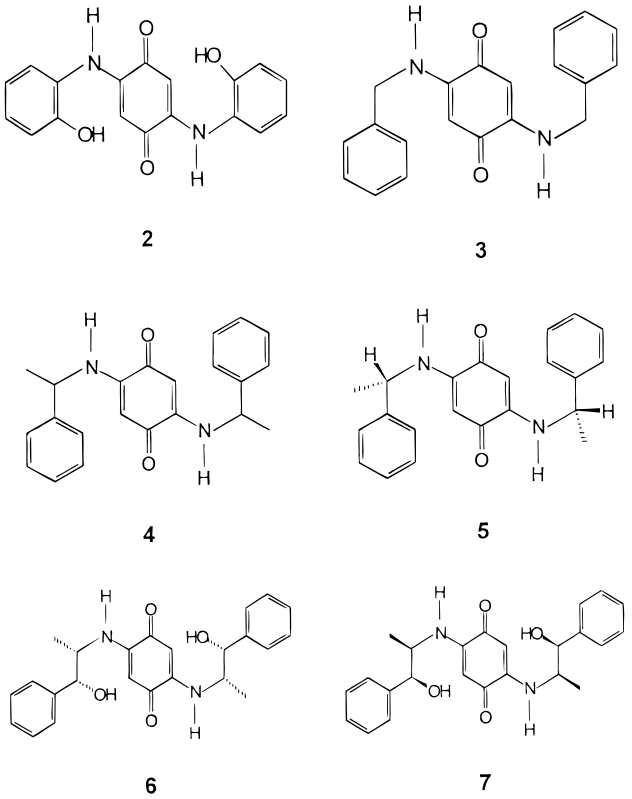


Figure 1. Chemical structures of 2,5-diamino-*p*-benzoquinones.

Sigma Chemical Co. Acetone, ethanol, and acetonitrile were from Merck. Acetonitrile was distilled from phosphorus pentoxide prior to use. Tetraethylammonium tetrafluoroborate (Et_4NBF_4) from Merck was used as supporting electrolyte and dried for 24 h at 60 °C before being used.

Apparatus. ^1H and ^{13}C NMR spectra were recorded using JEOL FX90Q and JEOL GSX-270 (270 MHz) spectrometers. Chemical shifts (ppm) are related to $(CH_3)_4Si$. DMSO-d_6, $CDCl_3$, and acetone-d_6 were used as solvents. Coupling constants are quoted in hertz. The HETCOR standard pulse sequence, which incorporates quadrature detection in both domains, was used as well. Infrared spectra were determined on a Perkin-Elmer 16 F spectrophotometer. Mass spectrometric analyses were carried out using a 5989A HP mass spectrometer coupled to a gas chromatograph 5890 series II (70 eV). UV spectra were determined on a Unicam SP 800 spectrophotometer. Melting points were obtained on a Gallenkamp MFB-595 apparatus and remain uncorrected. Electron transport was determined with an oxygraph YSI model 5300. pH changes were measured in a Corning pH meter model 12 attached to a Gilson recorder. Cyclic voltammetries were carried out on a BAS 100 B/W electrochemical analyzer with a 486 DX 33-MHz computer. Specific rotation was determined with a Perkin-Elmer 241 polarimeter.

Procedures for the Preparation of 2,5-Diamino-*p*-benzoquinones. *p-Benzoquinone* (**1**). UV (ethanol) λ_{max} (log ϵ) 242 (3.27) nm; log *P* 0.176. The concentration of **1** in the aqueous phase was 1.55 mM.

2,5-Di(o-aminophenol)-p-benzoquinone (**2**). *p*-Benzoquinone (5 g, 46.3 mmol) was dissolved in 350 mL of ethanol, and *o*-aminophenol (3.36 g, 30.7 mmol) was added. The solution was stirred at room temperature for 8 h. The solvent was evaporated to dryness, and the resulting solid was chromatographed over silica gel eluting with increasing polarities. The chloroform/ethanol (9:1) fractions afforded compound **2**, which was obtained as a red solid (2.02 g, 41%; mp 225 °C). Hydroquinone was obtained as byproduct and separated from the more polar fractions. ^1H NMR (DMSO-d_6, 270 MHz) δ 6.30 (s, 1H, H-3,6), 6.80 (s, 1H, OH), 7.34−7.42 (m, 3H, H-9,10, 11), 7.68 (d, 1H, J = 7.3 Hz, H-12), 8.60 (s, 1H, NH); ^{13}C NMR (DMSO-d_6, 67.8 MHz) 180.0 (C-1,4), 148.63 (C-2,5), 146.78 (C-8), 128.26 (C-7), 127.70 (C-11), 124.70 (C-12), 115.44 (C-10), 103.15 (C-9), 98.51 (C-3,6); IR (KBr) ν_{max} 3226 (OH), 3220 (NH), 1565 (C═O), 1513, 1507, 1457 cm^{-1}; UV (ethanol) λ_{max} (log ϵ) 434 (0.27); UV (octanol) λ_{max} (log ϵ) 342 (3.59) nm. Due to the fact that compund **2** was insoluble in water and slightly soluble in octanol, the partition coefficient could not be determined.

2,5-Di(benzylamine)-p-benzoquinone (**3**). A solution of *p*-benzoquinone (5.00 g, 46.3 mmol) and benzylamine (3.29 g, 30.7 mmol) in 350 mL of ethanol was stirred at room temperature for 4 h. The precipitate was filtered and washed with ethanol/chloroform mixtures (7:3) to yield 3.65 g (74%) of **3** as an orange solid: mp 253 °C; ^1H NMR (DMSO-d_6, 270 MHz) δ 4.36 (d, 2H, J = 6 Hz, CH_2-7), 5.10 (s, 1H, H-3,6), 7.22−7.35 (m, 5H, C_6H_5), 8.31 (t, 1H, J = 6 Hz, NH); ^{13}C NMR (DMSO-d_6, 67.8 MHz) δ 177.65 (C-1,4), 150.84 (C-2,5), 137.27 (C_{ipso}), 128.38 (C_{meta}), 127.08 (C_{ortho}), 127.03 (C_{para}), 93.1 (C-3,6), 44.98 (C-7); MS, *m/z* (%) 318 [M^+, 48], 241 (2), 213 (11), 130 (5), 91 (100), 65 (29), 39 (11); IR (KBr) ν_{max} 3278 (NH), 1552 (C═O), 1533, 1522, 1490, 1456, 1440, 1363, 1298, 1253 cm^{-1}; UV (ethanol) λ_{max} (log ϵ) 243 (4.42) nm; UV (octanol) λ_{max} (log ϵ) 347 (4.71) nm. Partition coefficient for compound **3** was not determined due to its insolubility in both water and octanol.

2,5-Di-(R,S-(+)-α-methylbenzylamino)-p-benzoquinone (**4**). To a solution of *p*-benzoquinone (12.97 g, 120 mmol) in 400 mL of ethanol was added (±)-α-methylbenzylamine (9.69 g, 80 mmol), and the solution was stirred for 8 h at room temperature. The solvent was evaporated to dryness, and the resulting solid was chromatographed over silica gel eluting with increasing polarities. Compound **4** was obtained from the hexane/ethyl ether (3:7) fractions as a red solid: mp 224 °C in 10.8% yield (1.50 g); the polar fractions afforded hydroquinone; ^1H NMR ($CDCl_3$, 270 MHz) δ 1.55 and 1.57 (d, 3H, J = 7.26 Hz, CH_3-8), 4.46 (quintet, 1H, J = 6.6 Hz, CH-7), 5.16 (s, 1H, H-3,6), 6.74 (d, broad, 1H, J = 6.6 Hz, NH), 7.19−7.37 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 178.70 (C-1,4), 149.60 (C-2,5), 141.66 (C_{ipso}), 128.96 (C_{meta}), 127.78 (C_{para}), 127.80 (C_{ortho}), 94.74 (C-3,6), 52.74 (C-7), 23.17 (C-8); MS, *m/z* (%) 346 [M^+, 65], 269 (2), 241 (22), 227 (23), 103 (21), 105 (100), 331 (24), 77 (32); IR (KBr) ν_{max} 3330 (NH), 3280, 3271, 1635 (C═O), 1569, 1540, 1517, 1494, 1452, 1351, 1295, 1227 cm^{-1}; UV (ethanol) λ_{max} (log ϵ) 341 (2.90) nm; UV (octanol) λ_{max} (log ϵ) 342 (2.90) nm; log *P* 0.982, the concentration of **4** in the octanol phase, 0.58 mM.

2,5-Di(R-(+)-α-methylbenzylamine)-p-benzoquinone (**5**). To a solution of *p*-benzoquinone (4.00 g, 37.0 mmol) in 300 mL of ethanol was added *R*-(+)-α-methylbenzylamine (3.00 g, 24.7 mmol), and the solution was stirred for 8 h at room temperature. The solvent was evaporated to dryness, and the resulting solid was chromatographed over silica gel eluting with increasing polarities. Compound **5** was obtained from the hexane/ethyl ether (3:7) fractions as a red solid: mp 190−191 °C in 14.5% yield (600 mg); ^1H NMR ($CDCl_3$, 270 MHz) δ 1.57 (d, 3H, J = 7.26 Hz, CH_3-8), 4.46 (quintet, 1H, J = 6.6 Hz, CH-7), 5.16 (s, 1H, H-3,6), 6.74 (d, broad, 1H, J = 6.6 Hz, NH), 7.19−7.37 (m, 5H, C_6H_5); ^{13}C NMR ($CDCl_3$, 67.8 MHz) δ 178.70 (C-1,4), 149.60 (C-2,5), 141.66 (C_{ipso}), 128.96 (C_{meta}), 127.78 (C_{para}), 125.80 (C_{ortho}), 94.74 (C-3,6), 52.74 (C-7), 23.17 (C-8); MS, *m/z* (%) 346 [M^+, 65], 269 (2), 241(22), 227 (23), 103 (21), 105 (100), 331 (24), 77 (32); IR (KBr) ν_{max} 3330 (NH) 3280, 3271, 1635 (C═O), 1569, 1540, 1517, 1494, 1452, 1351, 1295, 1227 cm^{-1}; $[\alpha]^{25}_D$ −1164 (*c* 0.010, ethanol); UV (ethanol) λ_{max} (log ϵ) 341 (2.90) nm; UV (octanol) λ_{max} (log ϵ) 342 (2.90) nm; log *P* 0.982. Partition coefficients for compounds **4** and **5** are identical, as expected, since they differ only in optical activity.

2,5-N,N-(1′R,2′R)-Norpseudoephedrine-p-benzoquinone (**6**). A solution of (−)-norpseudoephedrine (5.00 g, 33 mmol) and boric acid (681 mg, 11 mmol) dissolved in 75 mL of benzene was heated to reflux for 2 h and cooled to room temperature, and the solvent was evaporated to dryness. The resulting solid was dissolved in 75 mL of ethanol, *p*-benzoquinone (5.34 g, 49 mmol) was added, and the solution was stirred overnight at room temperature. The solvent was evaporated to dryness, and the resulting solid was chromatographed over silica gel using increasing solvent polarities. Compound **6** (5.5 g, 82%)

was separated from the chloroform/hexane fractions (50:50) as a red crystalline solid: mp 193−195 °C; ^{1}H NMR (acetone-d_6, 90 MHz) δ 7.62−7.30 (m, 5H), 7.0 (d, 1H, J = 8 Hz, NH) 5.33 (s, 1H, H-3), 5.00 (d, 1H, J = 8 Hz, H-5), 4.95 (m, 1H, H-4), 1.05 (d, 3H, J = 6 Hz, CH_3-4); ^{13}C NMR (acetone-d_6, 22.5 MHz) δ 178.8 (C-1), 151.8 (C-2), 143.6 (C_{ipso}), 127.3 (C_{ortho}), 129.2 (C_{meta}), 128.5 (C_{para}), 93.2 (C-3), 55.0 (C-4), 75.6 (C-5), 17.4 (CH_3); MS, *m*/*z* (%) 406 (M^+, 0.5), 300 (18), 193 (100), 177(32), 79 (54), 77 (43); IR (KBr) ν_{max} 1563 (C═O), 3300 cm^{-1}; $[\alpha]^{25}_D$ +849 (*c* 0.0155, ethanol); UV (ethanol) λ_{max} (log ϵ) 344 (2.86) nm; UV (octanol) λ_{max} (log ϵ) 346.5 (2.90) nm; log *P* 1.423, concentration of compound **6** in the octanol phase, 0.521 mM.

2,5-N,N-(1′S,2′R)-(+)-Norephedrine-p-benzoquinone (**7**). Synthesis was identical with that of **6** above. Compound **7** (6.00 g, 89%) was obtained from the chloroform/hexane (50:50) fractions as a red crystalline compound: mp 186−188 °C; ^{1}H NMR (acetone-d_6, 90 MHz) δ 7.60−7.25 (m, 5H, C_6H_5), 7.00 (d, 1H, J = 9 Hz, NH), 5.25 (s, 1H, H-3), 4.90 (d, 1H, J = 4 Hz, H-5), 3.72 (m, 1H, H-4), 1.38 (d, 3H, J = 6 Hz, CH_3-4); ^{13}C NMR (acetone-d_6, 22.5 MHz) δ 177.1 (C-1), 146.6 (C-2), 142.1 (C_{ipso}), 126.1 (C_{ortho}), 127.7 (C_{meta}), 126.9 (C_{para}), 92.2 (C-3), 52.9 (C-4), 73.0 (C-5), 13.4 (CH_3); MS, *m*/*z* (%) 406 [M^+, 1.1], 300 (33), 193 (100), 79 (30), 77 (27); IR (KBr) ν_{max} 1566 (C═O), 3330, 3263 (NH, OH) 2566 (C═O) cm^{-1}; $[\alpha]^{25}_D$ −848.0 (*c* 0.0155, ethanol); UV (ethanol) λ_{max} (log ϵ) 344 (2.86) nm; UV (octanol) λ_{max} (log ϵ) 346.5 (2.90) nm; log *P* 1.423, concentration of compound **7** in the octanol phase, 0.521 mM.

Biological Activity. Intact chloroplasts were prepared from market spinach leaves (*Spinacea oleracea* L.) as reported (Mills et al., 1980) and resuspended, unless indicated, in a solution containing 400 mM sucrose, 5 mM $MgCl_2$, and 10 mM KCl buffered with 30 mM Na^+-tricine at pH 8.0. KCN-poisoned chloroplasts were incubated with 30 mM KCN (pH 7.8) at 0 °C for 30 min (Izawa, 1977). Because incubations with cyanide are carried out with dilute thylakoid suspensions, the thylakoids were collected by centrifugation and resuspended in a smaller volume of cyanide-free solution. Light-induced, noncyclic electron transport in the presence of 2,5-diamino-*p*-benzoquinone derivatives (either MV, K_3[Fe(CN)], or DCPIP) as electron acceptor (Saha et al., 1971; Calera et al., 1995) was measured with an oxygen monitor using a Clark electrode attached to a YSI oxygraph model 5300 at 20 °C. KCN (0.1 mM) was added to inhibit any catalase activity. Stock solutions of 2,5-diamino-*p*-benzoquinone derivatives were prepared by dissolving these compounds in *N*′,*N*′-dimethylformamide; the final concentration of the organic solvent in the reaction mixture never exceeded 1%. Potassium ferricyanide is a useful electron acceptor that gives light-dependent oxygen evolution during photolysis of water by thylakoids. Another electron acceptor used for measuring whole-chain electrons utilizes the autoxidation of MV, which gives oxygen evolution (Allen and Hollmes, 1886). Reaction time of illumination was 1 min under aerobic conditions and saturating white light. Kinetic parameters were obtained by nonlinear regression analysis, using the Michaelis−Menten equation or the substrate inhibition equation when pertinent. The Sigma plot package was used to carry out the fitting procedure.

Extinction and Partition Coefficient Determinations. Extinction coefficients of *p*-benzoquinone and 2,5-diamino-*p*-benzoquinone derivatives were determined using the spectroscopic absorption technique (Fujita et al., 1964; Hansch et al., 1963). Partition coefficients were obtained by shaking the solute between 50 mL of 1-octanol and 50 mL of water, followed by spectrophotometric analyses of the octanol phases except for compound **1**.

Electrochemical Studies. The analyses were carried out using cyclic voltammetry as previously reported (Aguilar-Martínez et al., 1996); the cyclic voltammograms of 3 mM *p*-benzoquinone **3** and 2,5 diamino-*p*-benzoquinones **2**−**7** were obtained using a polished platinum disk electrode (area 2 mm^2) as the working electrode in 0.1 M Et_4NBF_4/acetonitrile as the electrolytic medium. The electroactivity range for this system was 3.8 V (from −1.8 to 2.0 V vs SCE). All measurements were performed at room temperature, and the scans were made at 100 mV/s.

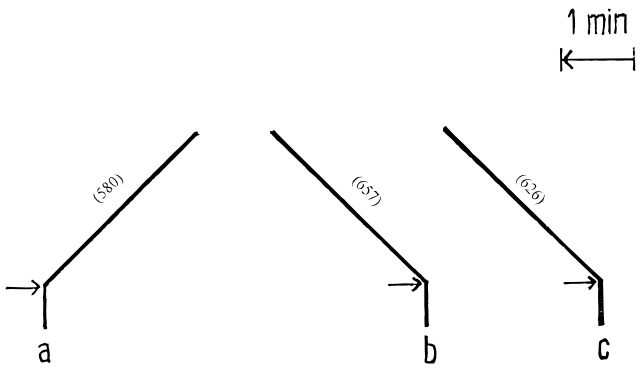


Figure 2. Polarographic traces showing the electron acceptor properties of compound **3** compared with MV and K_3[Fe(CN)$_6$]. Measurements were made by monitoring change of oxygen concentration in the medium using a Clark electrode attached to YSI oxygraph model 5300. The reaction mixture contained 0.1 M sorbitol, 5 mM $MgCl_2$, 10 mM KCl, 15 mM tricine−KOH buffer (pH 8.0), and thylakoids equivalent to 20 μg/mL chlorophyll; reaction volume, 3.0 mL; temperature, 20 °C: (a) Electron flow from water to 500 μM K_3[Fe(CN)$_6$] as oxygen evolution; (b) electron transport from water to 50 μM MV; (c) electron flow from water to 50 μM of compound **3**. Numbers on traces refer to mequiv e$^-$h^{-1} (mg of Chl)$^{-1}$. Arrows indicate onset of illumination.

RESULTS AND DISCUSSION

Synthesis. Compounds **2**−**7** (Figure 1) were prepared according to the method of Ross (1984) and were assayed as electron acceptors. Physical and spectroscopic constants of 2,5-diamino-*p*-benzoquinone and its derivatives are summarized under Materials and Methods. Compounds **2**−**5** were synthesized for the first time in this work. Compounds **6** and **7** were synthesized as described previously (Farfán and Contreras, 1985).

Biochemical Studies. If K_3[Fe(CN)$_6$] is used as electron acceptor in illuminated thylakoids (Figure 2a), oxygen is evolved from the photolysis of water (Allen and Holmes, 1986). However, if MV is used as electron acceptor at the whole electron transport chain of the chloroplast (see Figure 2b), oxygen is consumed (Allen and Holmes, 1986). Compound **3** stimulates oxygen uptake in illuminated thylakoids in analogy to MV (Figure 2c) and therefore accepts electrons from the electron transport chain in thylakoids. As pointed out by Hauska (1975), when chloroplasts are used in combination with electron donors and acceptors, they must not react with each other in a dark reaction. This trivial criterion is completely met by 2,5-diamino-*p*-benzoquinones, i.e., compound **3** (Figure 2). The data suggest that reduced compound **3** reacts with the O_2 of the medium producing $O_2^{\bullet-}$ in the same way as shown by reduced MV.

Photoreduction of compound **3** by illuminated thylakoids is given in Figure 3 as a function of increasing concentration of 2,5-diamino-*p*-benzoquinone **3** and measured as oxygen uptake. A concentration−response curve of this shape was typical for each 2,5-diamino-*p*-benzoquinone derivative. The photoreductions of K_3-[Fe(CN)$_6$], MV, and DCPIP are included as positive control.

Compounds **2** and **4**−**7** behave also as electron acceptors. The AC_{50} were obtained by kinetic analysis using the Michaelis−Menten equation or the substrate inhibition equation, and the results for all compounds tested are listed in Table 1. Compound **1** was used as control, since it is known that *p*-benzoquinone accepts electrons

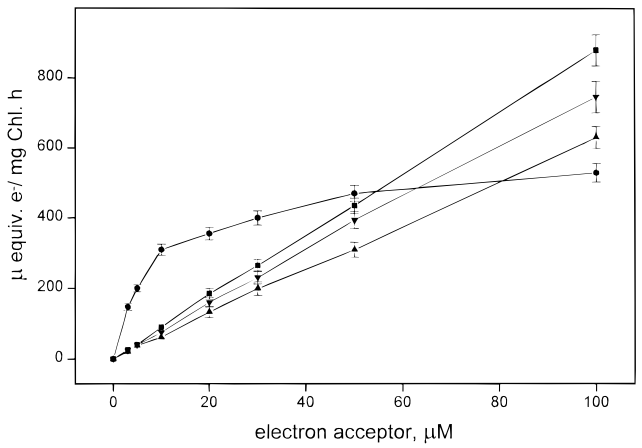


Figure 3. Rate of electron transport associated with the photoreduction of electron acceptors, compound **3** (●), methyl viologen (■), $K_3[Fe(CN)_6]$ (▲), and DCPIP (▼), by illuminated thylakoids. In each case a cuvette contained 20 μg of chlorophyll/mL. The reaction contained the following mixture and conditions: intact thylakoids (20 μg of chlorophyll/mL) were incubated in 3.0 mL of the following electron transport medium: 0.1 M sorbitol, 0.01 M KCl, 5 mM $MgCl_2$, 10 mM K-tricine (pH 8.0). Each point represents the mean of four determinations. Vertical bars on each curve represent the maximal value for standard deviation.

at the reducing side of PSII (Berg et al., 1980). Moreover, *p*-benzoquinone (**1**) allowed us to evaluate the effect of amine substitution at the 2- and 5-positions, thus, providing information on the redox potentials and biochemical studies. MV was also used as control for electron acceptor at the reducing side of PSI, and its sensitivity to inhibitors is compared with that of 2,5-diamino-*p*-benzoquinone derivatives. According to the AC_{50} (Table 1), the order of electron acceptance was **3** > **2** > **6** > **7** > **4** > **5**, compound **3** being the most powerful electron acceptor, due to the lower AC_{50} displayed. The AC_{50} values of compounds **4** and **5** were reached at higher concentrations than the other *p*-benzoquinone derivatives tested (Table 1). The saturation curve of compounds **2**−**7** was reached at different concentrations. These maximum concentrations were used in the experiments to locate the site(s) of action of compounds **2**−**7** in the electron transport chain of thylakoids (Table 2).

Further experiments were performed to determine the site where compounds **2**−**7** accept electrons using specific inhibitors of the electron transport chain, and adequate electron transport donors were included. DCMU and DBMIB (Izawa, 1977) inhibit uncoupled electron transport rates from water to compounds **2**−**7** (see Table 2); however, compound **1** was sensitive to DCMU and insensitive to DBMIB, indicating that it accepts electrons after Q_A and before cyt b_6f complex. These results indicate that compounds **2**−**7** accept electrons after PQ.

To further clarify the zone of electron acceptance for compounds **2**−**7**, PSI activity was measured with DCPIP/ascorbate as electron donors and MV as electron acceptors, and the results are shown in Table 3. It is observed that photoreduction of compounds **2**−**7** by thylakoids is inhibited by DCMU (Table 2), and it is reestablished after the addition of $DCPIPH_2$. The results are similar to those obtained when $DCPIPH_2$ was added to DCMU-inhibited thylakoids in the presence of MV. These data suggest that compounds **2**−**7** accept electrons in PSI or at least after cytochrome b_6f, since the photoreduction of compounds **2**−**7** can also be inhibited by DBMIB (Table 2).

To locate the site of electron acceptance of compounds **2**−**7**, thylakoids were poisoned with KCN, an electron transport inhibitor at the plastocyanin level (Izawa, 1977), since there is a stoichiometric release of plastocyanin Cu from the lamellar membrane exposed to KCN. Moreover, EPR spectroscopy confirmed the ability of reduced PMS to interact directly with P_{700} (Izawa et al., 1973). Therefore, we used 500 μM PMS/1000 μM ascorbate as the electron donor for P_{700}, MV as PSI electron acceptor, as well as 10 μM DCMU and 1 μM DBMIB to fully inhibit any electron flow prior to PC. Under these conditions, compounds **2**−**7** are still photoreduced by the electron transport span from P_{700} to the reducing side of PSI (Table 4), which suggests that compounds **2**−**7** accept electrons at/or after P_{700}, similarly to bipyridinium herbicides or dioxathiadiazaheteropentalenes, which accept electrons at the F_X level. The log *P* values of these 2,5-diamino-*p*-benzoquinone derivatives indicate that they have high hydrophobicity; therefore, they accept electrons at the hydrophobic side of the A_1 site of PSI (Camillieri, 1984, and references cited therein). It is noteworthy that the rate of electron flow from reduced PMS to **2**−**7** is lower than expected, due to the fact that PMS is a very good cyclic cofactor of PSI and also because it reacts with the oxygen of the medium.

Compounds **2**−**7** did not inhibit the Hill reaction at concentrations lower than those needed to reach AC_{50}. However, at higher concentrations (>AC_{50}) some of the quinones (**2**, **3**, and **7**) studied inhibited the Hill reaction. The basal electron transport rate in the presence of compounds **2**−**7** was accelerated by ADP plus P_i, or an uncoupler, and the phosphorylating electron flow was also accelerated by an uncoupler, as occurs with ferricyanide or MV (Table 2) in analogy to previous studies (Saha et al., 1971). Thylakoids used in this work were coupled, since control experiments using ammonium chloride (5 mM) induced 2- or 3-fold increases in the electron transport rate from water to MV. To find out whether electron transport from water to 2,5-diamino-*p*-benzoquinone derivatives is coupled to ATP formation, ATP synthesis was measured using compound **3** as electron acceptor. Figure 4 shows that the rate of ATP synthesis is directly proportional to the concentration of compound **3** up to 5 μM. Saturation of the system is reached at ≈20 μM of compound **3**. These data indicate that ATP formation is coupled to electron transport. The $P/2e^-$ ratio calculated from the rate of ATP synthesis and reduction of compound **3** was >1.00, which indicates that both photosystems are coupled to ATP synthesis, at low concentrations of the compound. However, as concentrations of compound **3** increase, the P/e_2 decreases (see the inset in Figure 4), which may indicate that the compound **3** has more than one action; that is, it inhibits H^+-ATPase, redox enzyme, or both. In Figure 4 is also included the P/e_2 of MV as control; the values obtained are similar to that reported by Saha et al. (1971).

Electrochemical Studies. To corroborate the site of electron acceptance for compounds **1**−**7**, their electrochemical properties were evaluated by cyclic voltammetry (scanned at 100 mV/s) of deoxygenated solutions in 0.1 M Et_4NBF_4 in acetonitrile. Half-wave reduction potentials [$E_{1/2} = (E_{pa} + E_{pc})/2$ (Heinze, 1984)]

Table 1. Half-Wave Potentials $E_{1/2}$ for Benzoquinone 1 and 2,5-Diamino-*p*-benzoquinones 2−7 Measured by Cyclic Voltammetry (100 mV/s vs SCE), 0.1 M Et_4NBF_4/Acetonitrile, and AC_{50} Data[a]

compd	$E_{1/2}$[b] (mV) vs SCE	i_{pa}/i_{pc}[b]	$E_{1/2E}$[c] vs SCE	i_{pa}/i_{pc}[c]	$E_{1/2}$[b] (mV) vs NHE	$E_{1/2}$[c] (mV) vs NHE	AC_{50} (μM)	K_i (mM)	V_{max}
1	−483	1.01	−1155	1.03	−239	−911	55.00		1520
2	−898	1.07	−1372	0.83	−654	−1128	0.65	0.101	309
3	−1049	1.02	−1536	0.97	−805	−1292	0.23		221
4	−999	0.95	−1457	0.87	−755	−1213	2.90		270
5	−1001	1.03	−1442	0.94	−757	−1198	15.40		
6	−991	0.91	−1234	0.79	−747	−990	0.68	1.75	290
7	−974	0.93	−1331	0.92	−730	−1087	0.84		382
MV									0

[a] AC_{50} is the concentration of the compound needed to accept 50% of electrons from the chloroplast electron transport chain. V_{max} in equiv e^- h^{-1} (mg of Chl)$^{-1}$. [b] Quinone/semiquinone redox couple. [c] Semiquinone/dianion redox couple.

Table 2. Inhibition of Electron Transport by DCMU and DBMIB with Compounds 1−7 and MV as Electron Acceptors

exptl conditions	BET	PET	UET	UET + DCMU	UET + DBMIB
MV	238	319	950	0	0
1	880	800	1520	0	1500
2	190	288	796	0	0
3	242	281	788	0	0
4	290	319	826	0	0
5	241	269	418	0	0
6	227	273	819	0	0
7	265	358	881	0	0

[a] Reaction mixture contained tricine buffer (pH 8.0), 10 mM; thylakoids with 20 μg of chlorophyll/mL; $MgCl_2$, 5 mM; KCl, 10 mM; sorbitol, 100 mM for basal electron transport (BET). For measurement of phosphorylating electron transport (PET) ADP, 1 mM and P_i, 3 mM, were added. for uncoupled electron transport (UET), NH_4Cl, 5 mM, was added. if used, DCMU, 10 μM; DBMIB, 1 μM. Rates are in μequiv e^- h^{-1} (mg of Chl)$^{-1}$. Saturation concentration of MV was 150 μM. Saturation concentrations of compounds **1**−**7** were 400, 10, 10, 150, 50, 60, and 3 μM, respectively.

Table 3. Uncoupled Photosystem I Electron Transport of 2,5-Diamino-*p*-benzoquinone Derivatives[a]

electron donor	electron acceptor	UET	UET + DCMU	UET + DCMU + $DCPIPH_2$
H_2O	MV	910	0	900
H_2O	**2**	796	0	790
H_2O	**3**	788	0	790
H_2O	**4**	826	0	820
H_2O	**5**	418	0	420
H_2O	**6**	819	0	820
H_2O	**7**	881	0	880

[a] Basal electron transport medium was used as reaction mixture as described in Table 2. Concentrations of electron acceptors as indicated in Table 2. If used, DCPIP/ascorbate, 100 μM/300 μM. Rates are in μequiv e^- h^{-1} (mg of Chl^{-1}).

are shown in Table 1. The $E_{1/2}$ value is a good approximation of the formal reduction potential E'_o for the quasi-reversible one electron reduction process of nonpolar *p*-benzoquinone and 2,5-diamino-*p*-benzoquinones. Under these conditions, all compounds were reversible (Fray, 1972) and stable radical anions were formed. The ratio of the anodic and cathodic peak currents was nearly equal to 1. It is well-known that in aprotic solvents the reduction reaction of quinones generally involves the following two succesive steps (Mann and Barnes, 1970):

$$Q + e^- \rightarrow Q^{\bullet -} \quad (1)$$

$$Q^{\bullet -} + e^- \rightarrow Q^{2-} \quad (2)$$

The behavior of the seven quinones studied here is consistent with the redox pathways outlined in reactions 1 and 2. The cyclic voltammogram for compound **3** showed two successive cathodic and anodic peaks (Figure 5). The first cathodic peak at −1100 mV corresponds to the reduction of Q to $Q^{\bullet -}$, and the anodic peak at −998 mV is consistent with the oxidation of $Q^{\bullet -}$ to Q ($E_{1/2}$ = −1049 mV). The reduction of $Q^{\bullet -}$ to Q^{2-} is observed at −1577 mV, while the oxidation of Q^{2-} to $Q^{\bullet -}$ is at −1494 mV ($E_{1/2}$ = −1536 mV) (Table 1).

Table 1 also shows that the introduction of aromatic amines and alkylamines as substituents on the *p*-benzoquinone system causes a shift in the half-wave potential to a more negative value compared to that of *p*-benzoquinone **1**. This can be explained by considering two separate electronic effects of the amino substituents. First, the inductive electron-withdrawing effect of the nitrogen atom, which always operates, regardless of the steric hindrance, i.e., if operated alone, would render reduction of 2,5-diaminobenzoquinones easier than reduction of 1,4-benzoquinone itself. Second, the resonance effect is generally predominant and results in delocalization of the lone pair of electrons of the nitrogen atom over the enone system of the quinone nucleus (as Scheme 1). This delocalization requires that the amino substituents can adopt a conformation in which the π-orbitals carrying the lone pair of electrons are parallel to the π system of the ring.

In quinone **2** with arylamino substituents at the C_2 and C_5 positions, the $E_{1/2}$ cathodic peak values for the

Table 4. Uncoupled Electron Transport from P_{700} to MV or 2,5-Diamino-*p*-benzoquinone Derivatives in KCN-Treated Thylakoids[a]

condition	electron donor	electron acceptor	rate of electron transport (% of control)
none control	H_2O	MV	100
KCN treated	H_2O	MV	8
KCN treated	PMS/ASC	MV	82
none control	H_2O	**3**	100
KCN treated	H_2O	**3**	9
KCN treated	PMS/ASC	**3**	85
KCN treated	PMS/ASC	**2**	84
KCN treated	PMS/ASC	**4**	80
KCN treated	PMS/ASC	**5**	85
KCN treated	PMS/ASC	**6**	84
KCN treated	PMS/ASC	**7**	82

[a] Reaction mixture as described in Table 2. Concentrations of MV and compounds **2**−**7** were 50, 5, 5, 100, 40, 30, and 20 μM, respectively. Control values for electron transport from 500 μM PMS/1000 μM ASC to 2,5-diamino-*p*-benzoquinone derivatives were **2**, 469; **3**, 438; **4**, 419; **5**; 438; **6**, 427; **7**, 519 μequiv e^- h^{-1} (mg of Chl^{-1}). Control value for electron flow from H_2O to MV (in KCN-treated thylakoids) was 96 μequiv e^- h^{-1} (mg of Chl^{-1}). Uncoupled electron transport rate from H_2O to **3**. H_2O to **3** (KCN treated), PMS/ASC to **3** (KCN treated) were 850, 76.5, 438 μequiv e^- h^{-1} (mg of Chl^{-1}), respectively. Other conditions as in Tables 2 and 3.

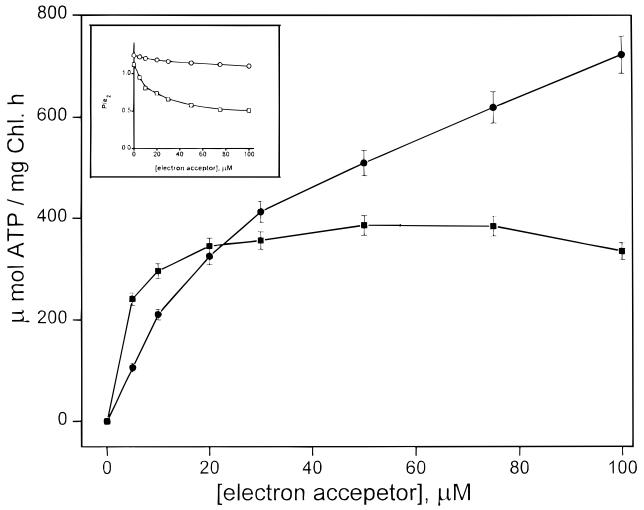


Figure 4. Photophosphorylation from water to MV (●) and to compound **3** (■) as electron acceptors in thylakoids isolated from spinach leaves. Photophosphorylation was measured in the presence of 1.0 mM ADP and 3.0 mM K_2HPO_4. The reaction contained the following mixture and conditions: thylakoids (20 μg of chlorophyll/mL) were incubated in 3.0 mL of the following electron transport medium: 0.1 M sorbitol, 0.01 M KCl, 5 mM $MgCl_2$, 10 mM K-tricine (pH 8.0). (Inset) P/e_2 represents the ratio of the number of molecules of ATP formed to the number of pairs of electrons transferred to the acceptors methyl viologen (○) and compound **3** (□).

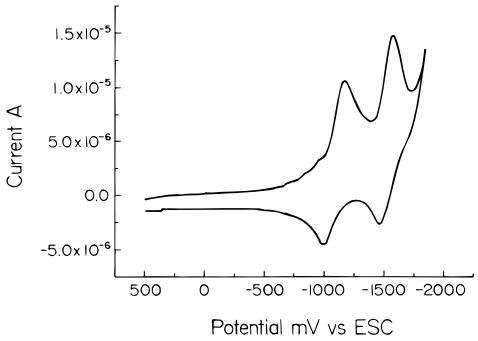
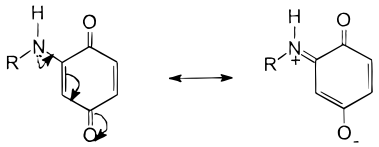


Figure 5. Cyclic voltammogram of **3** mM 2,5-di(α-methylbenzylamine)-*p*-benzoquinone containing 0.1 M Et_4NBF_4 in CH_3CN (working electrode Pt, reference SCE; scan rate 100 mV/s).

Scheme 1



first and second electron transfers are shifted to lower potential values than those of the alkylamine-*p*-benzoquinone derivatives **3**−**7** (Table 1). This can be attributed to the ortho steric effect exerted by the bulky hydroxy group in compound **2**, which is sterically hindered compared to *p*-benzoquinones **3**−**7** containing alkylamine substituents. The ortho steric effect in compound **2** could indeed be resposible for the loss of coplanarity of the amine group with the quinone ring, as shown in Scheme 1.

It was observed that *p*-benzoquinones having methyl groups in α-positions to nitrogen (compounds **4**−**7**) showed higher $E_{1/2}$ than **3** (Table 1). This may be due to the fact that the methyl groups hinder the molecular planarity, causing a shift to higher cathodic potentials. This steric hindrance is probably the major factor responsible for the appreciably higher half-wave potentials for compound **3** compared with those of compounds **4**−**7**. It is noteworthy that compounds **6** and **7**, having the same substituent chain but different stereochemistries, showed different $E_{1/2}$ values.

It is well-known (Iwaki and Itoh, 1989) that the midpoint potential values for the electron transport carriers at the reducing side of PSI, from A_0 to F_X, vary from −1.0 to −0.7 V vs NHE. Our results show that the capacity of 2,5-diamino-*p*-benzoquinone derivatives to accept electrons at the reducing side of PSI seem to be dependent on the first half-wave values for the $Q/Q^{\bullet-}$ couple ($E_{1/2}$) listed in Table 1, which vary between −0.805 and −0.654 V vs NHE (column 6). These redox potential values suggest that the *p*-benzoquinone derivatives accept electrons at the F_X site of PSI, which is in agreement with their activity in the electron transport chain of thylakoids. The low solubility of these compounds in water (refer to log *P* values under Materials and Methods) suggests that they accept electrons at the lipophilic site of the PSI where the phylloquinone A_1 to F_X are located.

Conclusions. The synthesized 2,5-diamino-*p*-benzoquinone derivatives **2**−**7** had appropriate $E_{1/2}$ potentials for the first wave to intercept electron transport at A_1 due to some degree of structural similarity or F_X level. Reduction of these 2,5-diamino-*p*-benzoquinones leads to formation of the anion radicals $Q^{\bullet-}$, which react rapidly with oxygen to yield superoxide $O_2^{\bullet-}$ and generate Q, in analogy to bipyridinium herbicides (Camilleri et al., 1984). The environment where *p*-benzoquinone derivatives accept electrons is in the hydrophobic domain of PSI, since the partition coefficients of these compounds showed that they are soluble in the octanol phase.

ABBREVIATIONS USED

DBMIB, 2,5-dibromo-3-methyl-6-isopropyl-*p*-benzoquinone; DCPIP, dichlorophenolindophenol; DCMU (diuron), 3-(3,4-dichlorophenyl)-1,1-dimethylurea; MV, methyl viologen or paraquat; PC, plastocyanine; PMS, phenazine methosulfate (*N*-methylphenazonium methosulfate); PSI, photosystem I; PSII, photosystem II; Q, quinone; $Q^{\bullet-}$, semiquinone radical anion; Q^{2-} quinone dianion; Q_A, primary quinone electron acceptor of photosystem II; Q_B, secondary quinone electron acceptor; PQH_2, reduced plastoquinone pool; SCE, saturated calomel reference electrode.

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