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The structure of streptonigrin semiquinone in solution [1]

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Abstract

Streptonigrin semiquinone ($SQ^{\cdot}$), a free radical intermediate implicated in the biological functioning of the antitumor antibiotic streptonigrin has been prepared and its structural properties in solution have been characterized. Through the use of electron paramagnetic resonance spectroscopy, the spin densities of the unpaired electron have been determined, indicating that the unpaired electron is largely confined to the quinolinesemiquinone moiety of the antibiotic. Unambiguous assignment of the hyperfine coupling constants was achieved employing isotopically labeled semiquinone radical, INDO molecular orbital calculations, and the study of unsubstituted 5,8-quinolinesemiquinone as a reference system. The assignments point to a negative spin density at the carbon para to the pyridine nitrogen in the radicals derived from both streptonigrin and the unsubstituted 5,8-quinolinequinone. Characterization of the properties of the streptonigrin semiquinone in solution indicate that the radical is stable in solution: it can be conveniently studied in 0.1 M methanolic lithium hydroxide or in aqueous organic solvent mixtures buffered with 0.06 M K_3PO_4 at pH 12.0. Under these conditions, the semiquinone shows completely reversible spectral changes between -10 to 60°C. Lowering the pH from 12.0 to 7.0 in aqueous DMSO decreases the lifetime of the radical from two weeks to a few minutes. Changes in structural properties of streptonigrin semiquinone in solution have been found to occur mainly due to variation in solvation and freedom of rotation of the amino group. Decreasing the temperature of $SQ^{\cdot}$ solution in methanol from 60 to -10°C leads to an increase in the hyperfine coupling constant to the amino nitrogen from 1.28 to 1.40 G, and those of the two amino protons from 0.73 and 0.73 to 1.02 and 1.11 G respectively, while the other coupling constants change less than 3%. Greater electron spin delocalization onto the -NH_2 group has been found throughout the solvent systems examined, yet the temperature at which

Abbreviations: EPR, electron paramagnetic resonance spectroscopy; $SQ^{\cdot}$, streptonigrin semiquinone; DMSO, dimethylsulfoxide; MeOH, methanol; SDS, sodium dodecyl sulfate; CTAB, hexadeclytrimethylammonium bromide; TTAB, tetradeclytrimethylammonium bromide; OG, octyl β-D-glucopyranoside; OSGP, octyl β-D-thioglucopyranoside

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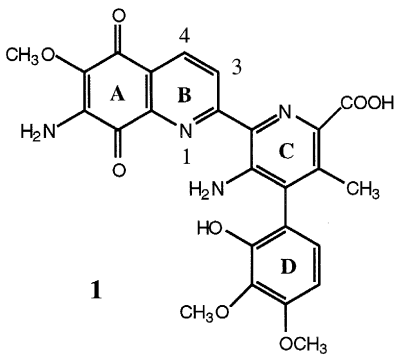
the two amino protons become equivalent changes with the nature of the solvent (e.g., from 25°C in MeOH to 40°C in aq. DMSO). The effective rotational diffusion constant as measured from EPR spectra in a series of solvents with fixed polarity (E_T) follows the Stokes–Einstein equation only in solvent mixtures of low to moderate viscosities (10–12.4 cP) suggesting that in addition to the viscosity of the medium a more specific mechanism (e.g., hydrogen bonding to the solvent) restricts the motion of the amino group. This hydrogen bonding mechanism is further supported by the fact that the degree of inequivalence in the amino proton hyperfine coupling constants varies monotonically with the spin density at the amino nitrogen. Studies of $SQ^{\cdot}$ in aqueous micellar dispersions using neutral, cationic, and anionic sufractants indicate that $SQ^{\cdot}$ is located at the surface of these aggregates. The structure of $SQ^{\cdot}$ changes little at neutral and anionic micellar surfaces; however, more severe structural changes occur in cationic micelles which appear to be consistent with a conformational change induced by the positively charged surface.

Keywords: Streptonigrin semiquinone; Quinolinesemiquinone; EPR; Structural property; Antitumor action; Micelle; Solvent dependence

1. Introduction

The aminoquinone antibiotic streptonigrin (**1**), a metabolite of *Streptomyces flocculus*, belongs to a group of antitumor antibiotics including mitomycin C, porfiromycin, antinomycin, rifamycin, and geldanamycin which possess the aminoquinone moiety [1,2].



Streptonigrin is one of the most effective agents for the treatment of cancers including lymphomas, melanomas, cancers of the breast, cervix, head and neck [3]. In addition to its anticancer activity, streptonigrin has been shown to possess a broad spectrum of antibacterial properties [4] and to display in vitro and in vivo antiviral activity [5] as well. The clinical use of streptonigrin has been precluded only because of its equally potent toxicity [6,7]. It has been suggested that reductive activation of streptonigrin results in formation of $SQ^{\cdot}$ that may interfere with depletion of cellular NADH/NADPH [1,4], uncoupling of oxidative phosphorylation and/or bring about single strand breakage of DNA [1,4,9].

Streptonigrin semiquinone appears to be the key intermediate in the biological functioning of this antibiotic [1,4–10]. Structural characterization of $SQ^{\cdot}$ is therefore an important prerequisite to subsequent elucidation of its redox chemistry which in turn is vital for understanding the chemical basis of its broad spectrum of biological activity. The functional role of $SQ^{\cdot}$ has been implicated in the mechanism of action of streptonigrin as it requires both a supply of intracellular electrons and the presence of oxygen [1,2]. As suggested, intracellularly reduced streptonigrin is reoxidized with concommittal formation of superoxide [11] and other active-oxygen derivatives (such as hydroxyl radicals) through the catalytic participation of metal ions [12–14] that are also essential cofactors for efficient funtioning of the drug [1,2,9,11–15]. Thus, $SQ^{\cdot}$ may act as a redox intermediate either in a single step one-electron reduction or in a series of two step sequential one-electron oxidations of the fully reduced dihydroquinone.

Notwithstanding the intense effort directed toward the total syntheses of streptonigrin, lavendamycin, streptonigrone, and preparation of other related analogues of the quinoline-5,8-dione ring system [15–19], it is the structural characterization of the redox-active intermediates such as the *p*-quinoline semiquinones that will likely provide the necessary mechanistic insight toward better understanding of the chemistry of this family of drugs [1,2].

Preparation and electron paramagnetic resonance spectroscopic (EPR) characterization of $SQ^{\cdot}$ under a

series of well-defined experimental conditions should provide important information not only with regard to the spin distribution and stablity of the semiquinone radical, but it should also shed light on the nature of interactions between the ring substituents and the media and how these interactions may respond to variation of the solvent parameters. Thus, in this paper, we describe the preparation and EPR characterization of the semiquinone in various solvents and as a function of temperature. Deuterium substitution and INDO calculations are employed to unambiguously assign the hyperfine coupling constants. The behavior of the radical associated with neutral, anionic, and cationic micelles is studied. The unsubstituted 5,8-quinolinesemiquinone is used as a model to separate competing inductive effects and hydrogen bonding interactions on the radical structure.

2. Materials and methods

2.1. Materials

Streptonigrin (NSC # 45383) was a generous gift of Dr. John. D. Douros, Drug Research and Development, Chemotherapy, National Cancer Institute. Spectrograde methanol and spectrograde dimethylsulfoxide were purchased from Burdick and Jackson and used as received. Methanol-d_4 and the following detergents were purchased from Sigma and used without further purification: sodium dodecyl sulfate, hexadecyltrimethylammonium bromide, tetradecyltrimethylammonium bromide, octyl β-D-glucopyranoside, octyl β-D-thioglucopyranoside, and Triton X-100. 1,4-Dioxane (99%) was purchased from Fisher and purified by passing through neutral alumina (J.T. Baker # 0537) before use. Deuterium oxide (99.8%) was purchased from ICN. The following materials were purchased from Matheson Coleman and Bell and used as received: ethylene glycol (98% by GC), 1,2-propanediol, lithium hydroxide monohydrate, sodium chloride, and ferric chloride hexahydrate. Lithium deuteroxide was prepared by deuterium exchange from 0.1 M $LiOH \cdot H_2O$ in D_2O followed by freeze drying. Deuteration was confirmed by ^{1}H-NMR. Sodium borohydride (98%, Stern Chemicals) and sodium borodeuteride (99% d, Chemical Dynamics Corporation) were used as received. 5-Amino-8-hydroxyquinoline dihydrochloride was purchased from Aldrich. *N*-Benzyldihydronicotinamide was prepared as described [20] and its structure and purity were confirmed by ^{1}H-NMR spectroscopy, thin-layer chromatography and UV absorption [8].

2.2. Methods

2.2.1. Preparation of streptonigrin semiquinone in methanol

Streptonigrin semiquinone ($SQ^{\cdot}$) was prepared in MeOH by dissolving 2.0 mg (4.0 μmol) of streptonigrin in 2.0 ml MeOH containing 0.1 M LiOH. To this solution was added solid (< 0.2 mg) $NaBH_4$ and the reaction mixture was deaerated by bubbling nitrogen gas through the solution for 2 min. The deep brown color of the quinolinequinone turned to dark green indicating the formation of the semiquinone. If a large excess of $NaBH_4$ was added, a yellow solution resulted that required extended exposure to air in order to obtain an EPR signal.

2.2.2. Preparation of deuterated streptonigrin seminquinone in d_4-methanol

The exchangeable hydrogen atoms in streptonigrin were deuterated in three ways. In the first two experiments, 2.0 mg (4.0 μmol)) streptonigrin were dissolved in 5.0 m/l of 1,4-dioxane-D_2O (1:1) and stirred for (a) 1 h and (b) 12 h. These solutions were then freeze dried. In the third method (c), streptonigrin was not pre-deuterated. Each of the above products was then dissolved in 2.0 ml of 0.1 M LiOD/MeOD to which solid $NaBD_4$ (< 0.2 mg) was added to generate the radical. The EPR spectra of the semiquinone obtained from each of the three deuteration methods were identical.

2.2.3. Preparation of semiquinone in other solvents

To study the solvent dependence of the hyperfine coupling constants, $SQ^{\cdot}$ was studied in other solvent mixtures and micellar solutions as follows: 10% DMSO in 0.06 M potassium phosphate buffer (pH 12.0); 50% 1,4-dioxane in 0.06 M potassium phosphate buffer (pH 12.0); 0.1 M detergent (pH 12.0) adjusted with LiOH (detergent = SDS, TTAB, CTAB, OG, OSGP, and Triton X-100). In each case, 2.0 mg of streptonigrin was added to 2.0 ml solution. Reduction was carried out by adding excess (< 0.2

mg) solid $NaBH_4$ or *N*-benzyldihydronicotinamide and samples were prepared as described above in MeOH. Deuterated $SQ^{\cdot}$ was prepared in the corresponding deuterated solvents using streptonigrin not previously deuterated. Other solvent mixtures employed (as summarized in Table 4) were prepared similarly.

2.2.4. Viscosity measurements

The viscosities of the solvent mixtures were measured using an Ostwald viscometer which was placed in a constant-temperature bath, and calibrated with distilled water. The densities of the solvent mixtures at various temperatures were determined with a Westphal balance.

2.2.5. INDO Calculations

All bond lengths, bond angles and dihedral angles, except for hydrogen, were taken from the published crystal structure [21]. Standard bond lengths and angles involving hydrogen atoms are taken from Pople and Beveridge [22]. The positions of rings C and D as well as the conformation of the amino group in ring A are unknown in solution and are not likely to be the same as in the crystal, but experimentally and theoretically the hyperfine coupling constants involving nuclei in rings C and D are negligible anyway. It was assumed that the structure of $SQ^{\cdot}$ was the same as the solid state structure of **1**. The CNINDO studies were carried out on a Cray-X-MP 24 Super Computer. The typical CPU time was approximately 60 s.

2.2.6. Spectral simulations

The EPR spectra were simulated using the algorithm of Oehler [23]. A stick diagram is generated according to the input hyperfine coupling constants and a line shape function applied to each stick at the end. The resulting simulated spectra were compared with experimental spectra and were optimized by trial and error. The best fitting line shape consisted of a linear combination of Lorentzian (30% doubly-integrated) and Gaussian each of the same linewidth [24]. In each spectral simulation a constant linewidth was employed for all of the lines; therefore, in more viscous solvents in which hindered rotation led to significant linewidth variation, the fits between the experimental spectra and the simulated spectra are poorer toward the high-field side of the spectra.

2.2.7. Preparation of the 5,8-quinolinesemiquinone

Ferric chloride oxidation of 5-amino-8-hydroxyquinoline dihydrochloride was used to synthesize 5,8-quinolinequinone according to the protocol by Pratt and Drake [25]. The UV absorption was measured in acetonitrile, 236.9 (20 000); methanol, 237.2 (19 500); and aq. phosphate buffer (pH 7.00), 240.1 (20 400). 0.6 mg of 5,8-quinolinequinone were added to 2.0 ml of the following solvent mixtures: aq. phosphate buffer (pH 7.40, 8.00, 10.00, and 12.00); deuterated aq. phosphate buffer pD 7.80; 0.1 M CTAB (pH 10.00 and 12.00); aq. phosphate buffer:DMSO (1:1) (pH 7.40 ± 0.02). For phosphate buffers of pH 8.00 or less, sodium borohydride (< 1 mg) or *N*-benzyldihydronicotinamide (< 1 mg) was added followed by air oxidation. The color changed from pale yellow to orange-brown. For phosphate buffers and CTAB solutions (pH 10.0 and above), no reducing agent was necessary to produce the seminquinone. The resulting samples from these latter solvents were dark olive-green. The DMSO solution buffered at pH 7.40 also required no reducing agent and slowly changed color from pale yellow to light brown.

2.2.8. EPR measurements

EPR spectra of the radicals were obtained with a Bruker 200 X-band spectrometer employing 100 kHz field modulation interfaced with an IBM 9000 computer. The field sweep was calibrated with an NMR gaussmeter. The spectra were obtained at 1.0 mW of microwave power incident upon the cavity. This power is near the limit of the linear plot of signal height versus square root of the microwave power, indicative of the absence of significant power saturation. A 12-G sweep in 500 s was employed with a time constant 0.32 s. Spectra were routinely run with smaller modulation amplitudes, time constants, and microwave powers in order to check for spectral distortion.

Samples were sealed into 50 μl disposable pipets and placed into a quartz dewar of our design fabricated by Wilmad as shown in Fig. 1. Odorless kerosene was thermostated to ± 0.05°C with a Neslab circulating bath and passed through the dewar. The temperature was measured with a thermocouple placed just above the active region of the cavity.

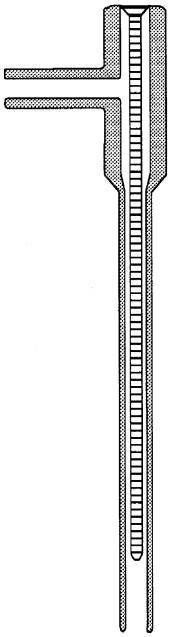


Fig. 1. Double-wall evacuated quartz dewar that fits inside of the microwave cavity for EPR experiments. The evacuated volume is indicated with dots. The cross-hatched region is a quartz tube, into which the sample capillaries fit, fused to the dewar at the top. Thermostated odorless kerosene is circulated through the dewar from the bottom to the top.

Temperature gradients were less than 0.05°C across the active part of the sample.

3. Results

3.1. Streptonigrin seminquinone in methanol at 25°C. Preparation and spectral assignments

Reduction of 2.0 mM streptonigrin in 0.1 M LiOH/MeOH with excess $NaBH_4$ followed by air oxidation produces a solution whose EPR spectrum is shown in Fig. 2a. When these solutions are kept sealed in capillary tubes, the signal persists for about one day; however, it disappears upon exposure to air in a few min. Streptonigrin semiquinone in MeOH can also be prepared with a concentration of LiOH as low as 2 mM yielding the same spectrum; however, the radical is less stable under less basic conditions. Alternatively, addition of solid *N*-benzyldihydronicotinamide as the reducing agent produces a solution that shows the same EPR spectrum.

The 18-line well-resolved spectrum shown in Fig. 2a appears intriguingly simple due to accidentally commensurate coupling constants. This same simplicity, however, leads to ambiguities in assignments of the hyperfine coupling constants. If we make no a priori assumptions about the number of interacting protons and nitrogen nuclei, 11 satisfactory combinations of coupling constants may be computer simulated.

In order to determine the correct combination, the exchangeable hydrogen atoms were deuterated and $SQ^{\cdot}$ was generated in MeOD/LiOD yielding the EPR spectrum shown in Fig. 3a. The spectrum was identical irrespective of the method of deuteration. This first-derivative spectrum is quite unusual, consisting of broad lines resembling a mixture of absorption and emission spectra. Interestingly, the unusual

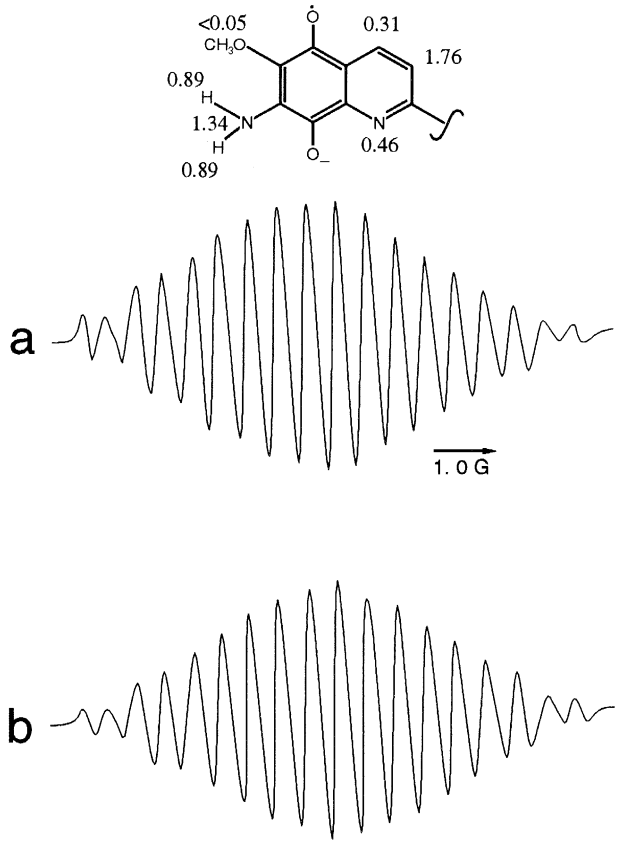


Fig. 2. (a) EPR spectrum of $SQ^{\cdot}$ in MeOH/LiOH at 25°C. Modulation amplitude, 0.078 G. Reduction of the modulation amplitude resulted in no further resolution. (b) Computer simulation using the parameters in the inset and in Table 1 with a linewidth of 0.18 G.

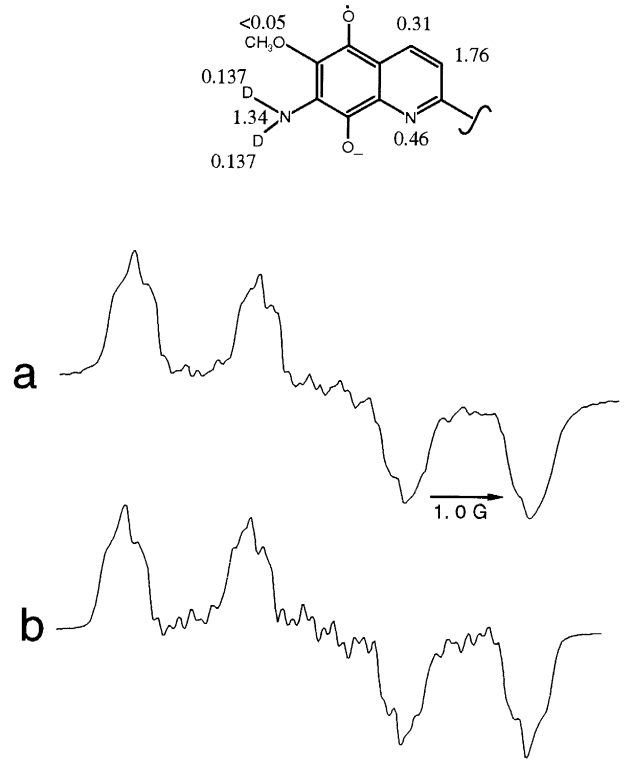


Fig. 3. (a) EPR spectrum of deuterated $SQ^{\cdot}$ in MeOD/LiOD at 25°C. Modulation amplitude, 0.031 G. Reduction of the modulation amplitude resulted in no further resolution. (b) Computer simulation using the parameters in the inset and in Table 1 with a linewidth of 0.13 G.

spectrum in Fig. 3a provided high selectivity in the choice of couplinling constants because the simulation of the spectrum turned out to be a very sensitive function of all of the parameters. Beginning with the 11 possible simulations consistent with Fig. 2a, and reducing two of the proton couplings by the ratio of the proton to deuteron gyromagnetic ratio, 6.51, while maintaining all of the other coupling constants, the linewidth, and line shape the same, unequivocally ruled out all of the possible assignments except one. This assignment is given in Table 1 and in the insets to Figs. 2 and 3. The absolute values of the coupling constants are given since these are derived from the EPR experiments. The simulations are shown in Fig. 2b and Fig. 3b.

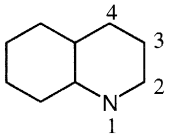
An ambiguity remained with respect to the assignments of a_3^H versus a_4^H corresponding to positions 3 and 4 of the pyridine ring. EPR studies of 9,10-anthrasemiquinone [26] and 1,4-dihydroxy-9,10-anthrasemiquinone [27] in both cases have shown that the coupling constant to the proton at carbon 7 (comparable to our position 3) is greater in magnitude than that at carbon 8 (comparable to our position 4) in agreement with the assignments presented in Table 1; however, the signs of the couplings are not known. Further, the presence of the pyridine nitrogen complicates the present situation. Therefore, INDO calculations were carried out and are given in Table 1. These calculations do show a larger magnitude coupling to the proton at position 3. In addition, since the calculations yield the signs of the coupling constants, they show that the sign of the coupling to the proton at position 4 is positive. In π radicals, the hyperfine coupling constant of a proton attached to a carbon is of opposite sign to the spin density upon the carbon [28], thus, taking the INDO calculations as correctly giving the sign of the coupling constants, the spin density is positive at carbon 3 and negative at carbon 4.

The observed g-value, $g = 2.0043 \pm 0.0001$, is comparable to values found in other semiquinone radicals; for example, $g = 2.0044$ in 5,8-dimethoxynaphthoquinone [29], $g = 2.0043$ in 6-chloro-8-hydroxy-5-methoxy-1,4-naphthoquinone [29], and $g = 2.0044$ in 9,10-anthrasemiquinone [26]. Comparison of the coupling constants with those

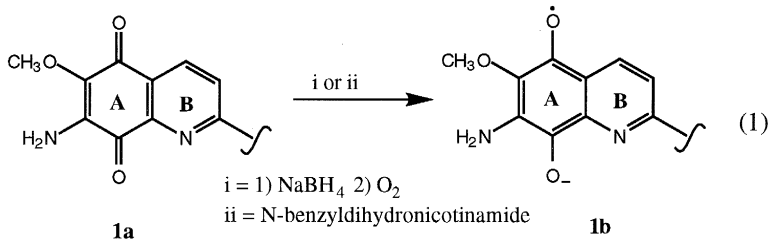
Table 1
Hyperfine coupling constants (Gauss) for streptonigrin semiquinone and deuterated streptonigrin semiquinone in basic methanol

Coupling	Experimental [a]	INDO Streptonigrin	INDO Compound **2**
$a^N_{NH_2}$	1.34	0.27	0.19
$a^H_{NH_2}$	0.89	0.62 [b]	0.83 [b]
$a^D_{ND_2}$	0.137		
$a^H_{OCH_3}$	< 0.05	−0.17	−0.10
a^H_4	0.31	0.58	0.61
a^H_3	1.76	−1.10	−0.90
a^N_1	0.46	0.05	0.12

$T = 25$°C.
[a] Absolute values are derived from the EPR analysis. [b] Average value of the two proton couplings. $g = 2.0043 \pm 0.0001$.



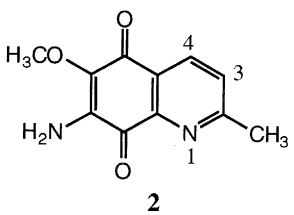
reported for synthetic aminosemiquinones [30] and related heterocyclic semiquinones [31] leaves little doubt that the radical is indeed the 5,8-quinoline-semiquinone, **1b**.



The lack of a hydroxyl proton coupling constant shows, as is expected under basic conditions, the absence of that proton in the semiquinone.

The fact that the methoxy couplings are negligible is noteworthy since these couplings seem to vary in related radicals depending upon the nature of the ring substituents [30,32–34]. The equivalent exchangeable couplings which are assigned to the amino hydrogen atoms are comparable to those in related systems reported in the literature [35,36]. The small nitrogen coupling assigned to the heterocyclic nitrogen is comparable to such couplings in related systems [31]. The hyperfine coupling constants in $SQ^{\cdot}$ are consistent with a pattern established earlier [37] that in heterocyclic semiquinones, the ring containing the nitrogen atom has very little effect on the spin density.

The fact that we observe no significant hyperfine couplings to nuclei in rings **C** and **D** shows that minimal spin density resides upon those rings. Indeed, it is expected that the presence of rings **C** and **D** would have a small effect upon the spin distribution in rings **A** and **B**. To investigate this point theoretically, INDO calculations were carried out for semiquinone of the model compound, **2**.



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The results of these calculations, given in the final column of Table 1, show that the couplings in $SQ^{\cdot}$ and the semiquinone of compound **2** are similar.

3.2. Temperature dependence of the EPR spectra

The temperature dependence of the EPR spectrum of $SQ^{\cdot}$ in methanol is shown in Fig. 4 together with the simulations. At temperatures both above and below 25°C, the highly symmetric spectrum gives way to spectra of lower symmetry. The variations of the

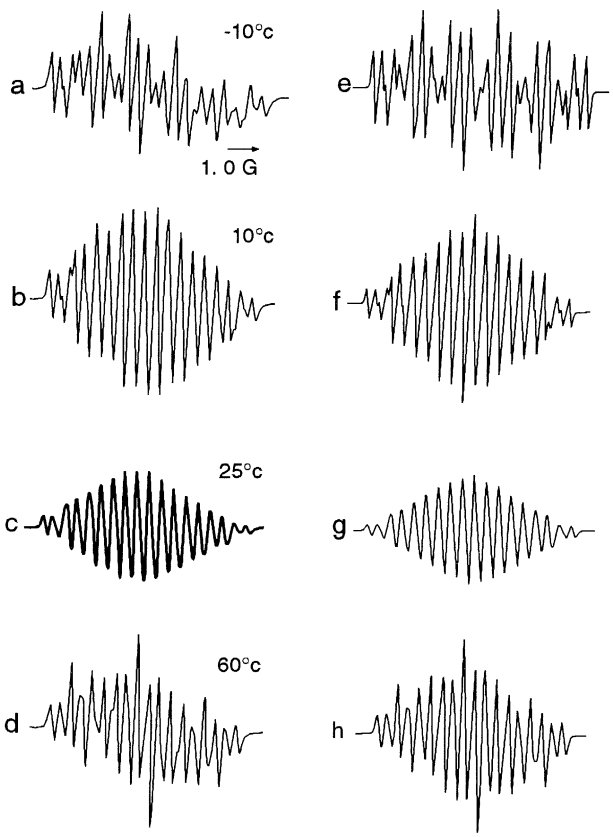


Fig. 4. (a)–(d) EPR spectra of $SQ^{\cdot}$ in MeOH/LiOH at various temperatures. Modulation amplitude, 0.12 G. (e)–(h) Computer simulations using the parameters in Table 2 with linewidths as follows: (e) 0.13 G, (f) 0.13 G, (g) 0.18 G, and (h) 0.18 G.

Table 2
Hyperfine coupling constants (Gauss) versus temperature. Streptonigrin semiquinone in methanol

T (°C)	a_3^H	a_4^H	$a_{NH_2}^N$	$a_{NH_2}^H$	$a_{NH_2}^H$	a_1^N
−10	1.78	0.31	1.40	1.11	1.02	0.46
−5	1.78	0.31	1.40	1.07	1.00	0.46
0	1.78	0.31	1.39	1.03	0.97	0.46
5	1.77	0.31	1.38	1.00	0.94	0.46
10	1.77	0.31	1.37	0.97	0.92	0.46
15	1.76	0.31	1.36	0.95	0.91	0.46
20	1.76	0.31	1.35	0.92	0.88	0.46
25	1.76	0.31	1.34	0.89	0.89	0.46
30	1.75	0.31	1.33	0.86	0.86	0.45
35	1.75	0.31	1.32	0.84	0.84	0.45
40	1.75	0.31	1.31	0.82	0.82	0.45
45	1.74	0.31	1.30	0.80	0.80	0.45
50	1.74	0.31	1.29	0.78	0.78	0.45
55	1.73	0.31	1.28	0.75	0.75	0.45
60	1.72	0.31	1.28	0.73	0.73	0.45

hyperfine coupling constants with the temperature are given in Table 2. All of the changes with temperature were reversible and most of the spectral changes are attributed to the temperature variation of the hyperfine coupling constants due to the amino group. Other than the amino group, the hyperfine coupling constants changed by less than 3% on raising the temperature from −10 to 60°C. The amino nitrogen coupling constant decreased by about 9% over this temperature range. The amino proton hyperfine coupling constants showed an even more substantial variation; upon increasing the temperature from −10 to 60°C, the $a_{NH_2}^H$ values decreased by more than 30%. Furthermore, it should be noted that the amino proton coupling constants that were found to be equivalent at 25°C become inequivalent at lower temperatures.

3.3. Solvent dependence of the EPR spectra

In order to examine the role of solvation in influencing the spin density distribution and conformation of streptonigrin semiquinone, we have studied two types of solvent mixtures: (1) aqueous buffers mixed with organic solvents (dioxane, dimethyl sulfoxide) to obtain homogeneous solutions of the water-insoluble antibiotic and (2) micellar dispersions of anionic, neutral, and cationic sufractants in order to assess the role of solute–solvent interactions between the surfactant micelles and the negatively charged radical.

The EPR spectra of $SQ^{\cdot}$ in 10% DMSO in aqueous 0.06 M potassium phosphate buffer (pH 12.0) and in deuterium oxide are presented in Fig. 5a and c, respectively. The corresponding simulated spectra are given in Fig. 5b and d. The hyperfine coupling constants and assignments are given in Table 3 and the inset to Fig. 5. Despite the remarkable difference in the appearance of spectra due to $SQ^{\cdot}$ in methanol, Fig. 2, and dimethylsulfoxide, Fig. 5, the hyperfine coupling constants turned out to be quite similar. The amino nitrogen coupling increases by 8% on moving from MeOH to aqueous DMSO. All other coupling constants are less affected; however, it is of interest that the two amino protons that are equivalent in MeOH at 25°C are inequivalent in DMSO at the same temperature. Spectra similar to those shown in Fig. 5 are observed when $SQ^{\cdot}$ is prepared in 50% 1,4-dioxane 0.06 M potassium phosphate buffer (pH 12) and the corresponding hyperfine coupling constants are also included in Table 3. These assignments were consistent with the spectra observed for the deuterated streptonigrin semiquinone.

The spectra of $SQ^{\cdot}$ in the anionic micellar dispersions obtained in 0.1 M aqueous SDS at 5, 25, and 50°C and the computer simulations are shown in Fig. 6. At this detergent concentration, approximately spherical micelles [38] containing about 70 molecules [39] are formed, leading to approximately a 1.3 mM concentration of micelles. Since streptonigrin is only sparingly soluble in water, it is most likely associated with the micelle. As additive molecules, especially those containing hydrophilic moieties such as $SQ^{\cdot}$ are generally located in the interfacial region of micelles [40], the radical is expected to be at the micellar interface. Noteworthy is the fact that the linewidths in the micellar environment were found to be comparable with those in homogeneous solvents suggesting that the radical tumbles quite rapidly under the influence of a rather low local viscosity [3]. The hyperfine coupling constants and assignments of $SQ^{\cdot}$ are given in Table 3 indicating that the major difference is in increasing the coupling constant of the amino nitrogen by about 10% on going from MeOH to aq. SDS. Furthermore, the highly symmet-

[3] Solubilized nitroxide free radicals derived from fatty acids show similar rapid rotational motion in SDS micelles [41].

Table 3
Hyperfine coupling constants (Gauss) for streptonigrin semiquinone in a series of solvent systems

Solvent system		T (°C)	a_3^H	a_4^H	$a_{NH_2}^N$	$a_{NH_2}^H$	$a_{NH_2}^H$	a_1^N
Solvents								
MeOH		5	1.77	0.31	1.38	1.00	0.94	0.46
		25	1.76	0.31	1.34	0.89	0.89	0.46
		60	1.72	0.31	1.28	0.73	0.73	0.45
10% DMSO/H_2O		25	1.73	0.27	1.45	0.89	0.83	0.47
50% 1,4-Dioxane/H_2O		25	1.75	0.26	1.39	0.80	0.77	0.43
Micelles [a]								
	Charge							
SDS	−	5	1.76	0.25	1.52	0.97	0.88	0.50
		60	1.72	0.25	1.41	0.68	0.68	0.45
Triton X-100	0	5	1.78	0.27	1.52	0.97	0.88	0.48
		60	1.73	0.25	1.41	0.68	0.68	0.42
OG	0	5	1.77	0.25	1.52	1.01	0.88	0.48
		60	1.73	0.25	1.40	0.68	0.68	0.44
OSGP	0	5	1.77	0.26	1.52	0.99	0.87	0.49
		60	1.73	0.26	1.40	0.68	0.68	0.45
CTAB	+	5	1.59	0.19	1.15	0.85	0.77	0.36
		25	1.59	0.22	1.24	0.75	0.68	0.35
		60	1.60	0.24	1.34	0.53	0.53	0.33
TTAB	+	25	1.54	0.20	1.25	0.72	0.68	0.33

[a] See abbreviations.

ric spectrum observed in MeOH at 25°C is not found at any temperature in aqueous SDS. As in other solutions, all of the changes with temperature were reversible and most of the spectral changes can be attributed to the temperature dependence of the hyperfine coupling constants of the amino group. It should be pointed out that in contrast with methanol, the amino proton hyperfine coupling constants in SDS are inequivalent at 25°C, becoming equivalent at temperatures above 40°C.

The spectra and temperature dependence spectrum of $SQ^{\cdot}$ in aqueous dispersions of OG, OSGP, and Triton X-100 which form neutral micelles are very similar to those in the anionic SDS micelles as shown in Table 3. Remarkably, in all of the neutral micelles as in SDS, the amino proton hyperfine coupling constants are inequivalent at 25°C and become equivalent at temperatures above 40°C.

In constrast to the neutral and anionic micellar dispersions, in the cationic micellar solutions formed by TTAB and CTAB, the spectra of $SQ^{\cdot}$ are rather different; this is especially apparent in the deuterated preparations. For example, Fig. 7 shows the spectra of $SQ^{\cdot}$ in CTAB at 60°C. The deuterated radical displays rather broad lines, but they have the character of normal first-derivative spectra rather than the odd shaped spectra of Fig. 3a and Fig. 5c. The linewidths of the spectra are significantly broader at a given temperature in the cationic micelles than they are in non-micellar solutions, SDS micelles, or the neutral micelles indicating a more severely hindered rotation at the surface of the cationic aggregates [42]. Indeed, this is not unexpected for a negatively charged radical at the surface of a positively charged micelle. The experimental parameters confirmed by computer simulation are given in Table 3. Comparing the coupling constants in TTAB versus CTAB micelles shows that the structure of $SQ^{\cdot}$ is insensitive to the chain length of the surfactant. This provides additional evidence that the radicals are very likely located in the interfacial region [40].

We have also performed some brief experiments that show that adding salt to the micellar solutions in order to increase their sizes [43] does not influence the semiquinone structure. For example, in the case of SDS, in the presence of 0.5 M NaCl which increases the aggregation number of the micelle from about 70 molecules to about 600 molecules [44], the EPR spectrum of $SQ^{\cdot}$ remained unchanged. Similarly, no changes in the spectra due to $SQ^{\cdot}$ were

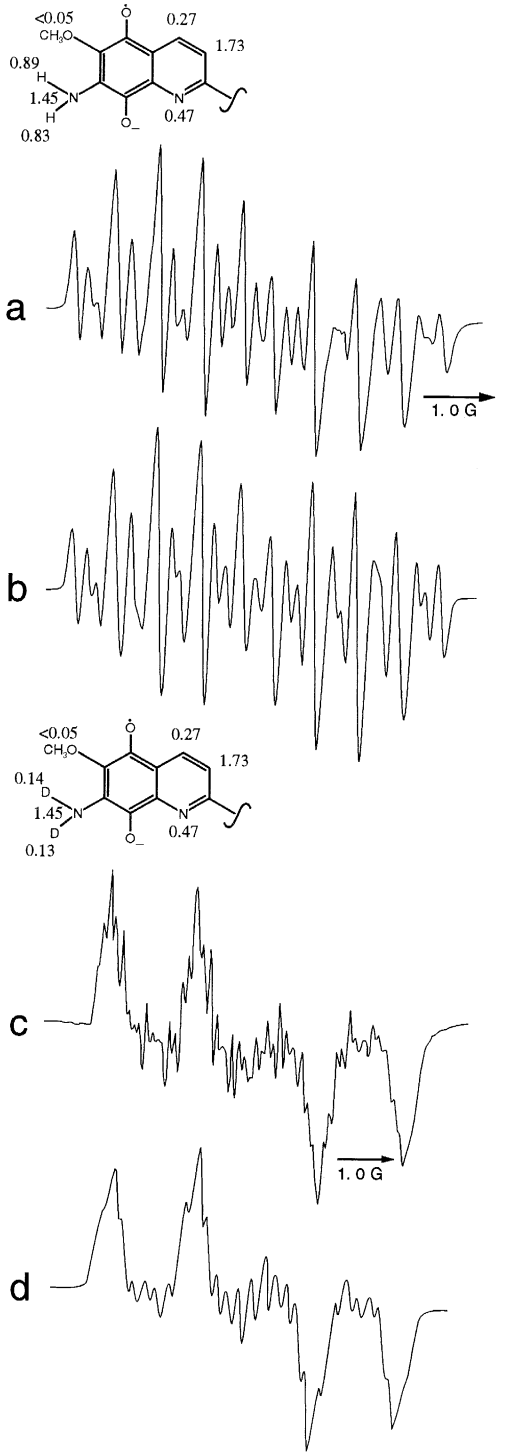


Fig. 5. EPR spectra of $SQ^{\cdot}$ in (a) $DMSO/H_2O$ and (c) $DMSO/D_2O$ at 25°C. Modulation amplitude, 0.078 G. (b) and (d) Computer simulations using the parameters in the inset and in Table 3 with a linewidth of 0.18 G.

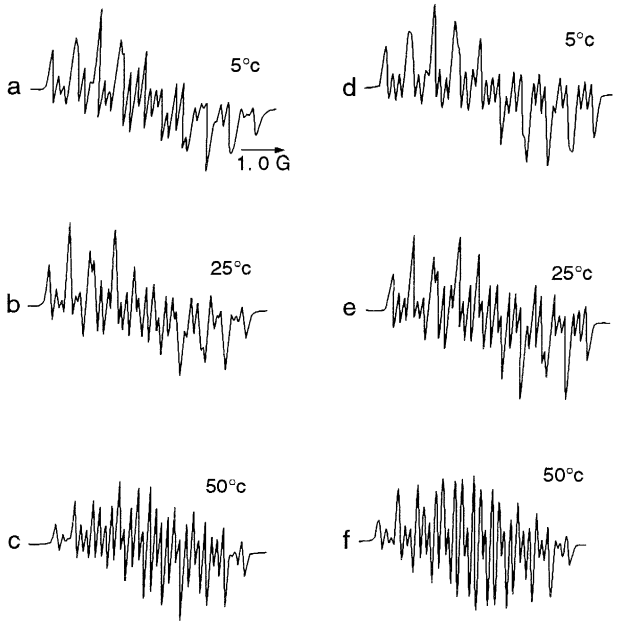


Fig. 6. (a)–(c) EPR spectra of $SQ^{\cdot}$ in SDS at various temperatures. Modulation amplitude, 0.078 G. (d)–(f) Computer simulations using the parameters in Table 3 with a linewidth of 0.18 G.

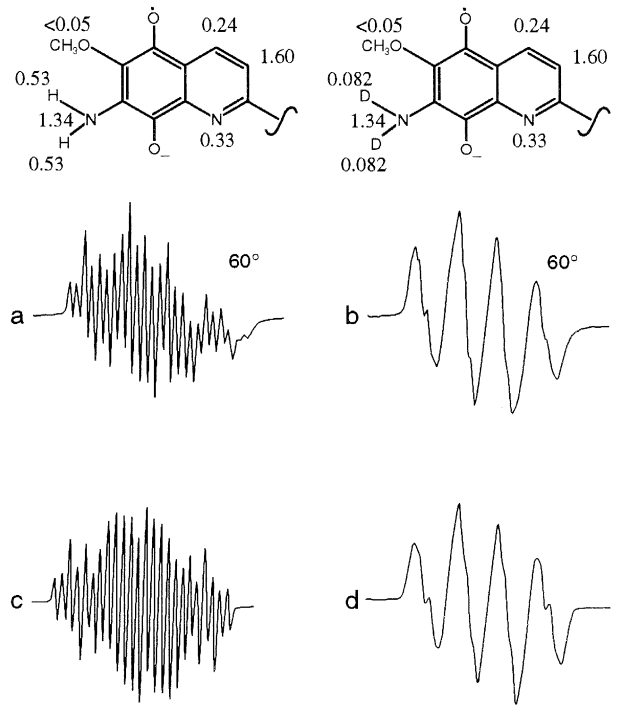


Fig. 7. EPR spectra of $SQ^{\cdot}$ in CTAB in (a) $H_2O/LiOH$, modulation amplitude, 0.062 G and (b) $D_2O/LiOD$, modulation amplitude, 0.039 G. Computer simulations using the parameters in the insets and in Table 3 with a linewidth of (c) 0.12 and (d) 0.24 G.

observed in response to the addition of salt in CTAB and Triton X-100 micelles.

3.4. Separating the effect of solvent polarity and viscosity on the EPR spectra

The EPR spectra of $SQ^{\cdot}$ vary with both temperature and the nature of the solvent. To address the question whether these variations are specific to the solvent or are related simply to the polarity and viscosity of the medium we have examined the effect of viscosity on the spectra. To characterize the polarity, we have chosen the E_T-value [45], a microscopic solvent polarity parameter, as being more appropriate to assess the local solvation of the radical than the bulk dielectric constant [46]. In order to obtain media of constant polarity, we have prepared a series of solvent mixtures with fixed E_T values as shown in Table 4. The composition of these mixtures were determined by interpolation from literature values [45]. We then measured the viscosity of each solution corresponding to a specific fixed polarity.

To analyze the results, we have taken advantage of the fact that most of the temperature dependence of the EPR spectra is due to the amino group. The temperature dependence is certainly due to rotational reorientation of the $-NH_2$ group with respect to the quinolinesemiquinone ring. If we model the portion of the rotational diffusion constant that is due to hydrodynamic drag provided by the medium as a Stokes–Einstein equation,

$$D = kT/\xi \quad (1)$$

where D is the rotational diffusion constant, k the Boltzmann constant, and ξ is the friction constant. In turn the friction constant ξ is proportional to η, where η is the shear viscosity of the medium with a constant of proportionality depending upon the details of the motion. Thus conformity to a Stokes–Einstein model of rotational diffusion is manifest by a diffusion constant that varies as T/η as given in Eq. (2).

$$D = \text{constant}\,T/\eta \quad (2)$$

where the constant would depend upon the details of the motion. For a good discussion of the Stokes–Einstein equation see Ref. [47] and references within. Irrespective of the details of the motion (the value of the constant in Eq. (2)), we reason that for a given set of hyperfine coupling constants (a given spectrum), the motion of the amino group must be similar. Therefore, finding the same hyperfine coupling constants for the same value of T/η would imply that the Stokes–Einstein equation, Eq. (2), held which in turn would mean that nonspecific viscous drag of the medium dominated the motion.

In order to test if the motion of the amino group follows the Stokes–Einstein equation, Eq. (2), we searched by trial and error, for temperatures that gave identical spectra in solvent mixtures of the same polarity (E_T). This was successful. Spectra that overlay one another in every detail could be produced and the temperatures at which this occurred are given in the third column of Table 4. The experimentally determined viscosities are given in the fourth column of Table 4 together with the calculated T/η ratios in the fifth.

Examination of the data in Table 4 reveals that, with the polarity fixed, the Stokes–Einstein equation holds approximately at lower viscosities, varying only about 5% from the mean for the solvent mixtures in the 10–12.4 cP range (with $E_T = 61.2$ kcal/mol); however, for the higher viscosity mixtures 13.2–23.2 cP (with $E_T = 58.6$), this variation reached almost 30%. It appears therefore that in addition to the effect of viscosity, specific solute-solvent interactions be-

Table 4
Solvent mixtures [a] with the same E_T-values

E_T (kcal/mol)	Solvent mixture	T (°C)	η (cP)	T/η (K/cP)
61.2	10% 1,1-dioxane	25 [b]	10.0	27.1
61.2	10% 1,2-propanediol	27 [b]	11.7	25.6
61.2	28% ethylene glycol	35 [b]	12.4	24.8
58.6	20% 1,1-dioxane	25 [c]	13.2	22.6
58.6	30% 1,2-propanediol	30 [c]	19.5	15.6
58.6	56% ethylene glycol	42 [c]	23.3	14.2
57.1	30% 1,1-dioxane	25 [d]		
57.1	62% 1,2-propanediol	32 [d]		

[a] Organic solvent mixed with phosphate buffer (pH 12.0). [b] Temperature at which three solvent mixtures with $E_T = 61.2$ kcal/mol gave identical spectra. [c] Temperature at which three solvent mixtures with $E_T = 58.6$ kcal/mol gave identical spectra. [d] Temperature at which two solvent mixtures with $E_T = 57.1$ kcal/mol gave identical spectra.

tween the radical and the solvation shell play a role in the EPR spectra of $SQ^{\cdot}$.

3.5. Stability of $SQ^{\cdot}$ versus pH

When streptonigrin semiquinone was prepared in 50% aq. DMSO under less basic conditions (aq. 0.06 M potassium phosphate (pH 7.0) containing 10% DMSO), the radical produced an identical EPR spectrum as in more basic conditions, but it was found to be less stable. Specifically, at pH 7.0, the signal decayed in a few minutes, while at pH 12, in aq. DMSO and SDS micellar dispersions as well, it persisted for about two weeks in sealed capillary tubes. These observations seem to indicate that under less basic conditions, the radical probably decays via disproportionation to give the dihydroquinone and the quinone, consistent with the observation reported in the literature for disproportionation of ortho-semiquinone radical at pH 7.0 and 5.0 to give diols and *o*-quinones [48].

3.6. 5,8-Quinolinesemiquinone

The EPR spectrum of 5,8-quinolinesemiquinone in DMSO 0.06 M potassium phosphate buffer (pH 7.4) at 25°C is shown in Fig. 8. The twenty-four line spectrum was interpreted as given in the inset to Fig. 8 and the parameters are given in Table 5 together with parameters derived from other solvent systems. The assignments of hyperfine coupling constants to the protons at positions 6 and 7 are made by analogy

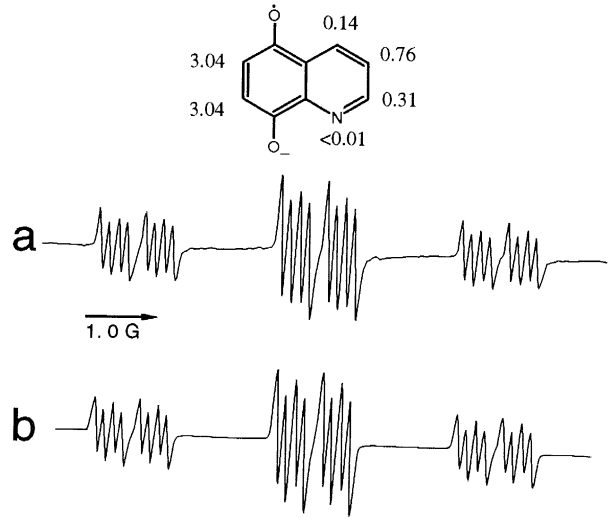


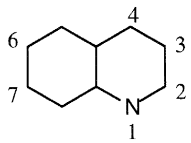
Fig. 8. (a) EPR spectrum of 5,8-quinolinesemiquinone in DMSO/phosphate buffer (pH 7.40) at 25°C. Modulation amplitude, 0.039 G. (b) Computer simulation using the parameters in the inset and in Table 5 with a linewidth of 0.09 G.

with 1,4-naphthosemiquinone anion [35] and various anthrasemiquinones [26] in which proton couplings in the quinone ring are in the range 3.40 to 2.95 G. The next largest coupling of magnitude 0.78 G is assigned to position 3 in analogy with the assignment of the largest proton coupling in $SQ^{\cdot}$. This means that the spin density in the pyridine ring of 5,8-quinolinesemiquinone is only about 40% of that in $SQ^{\cdot}$. There are two more proton coupling constants which we have tentatively assigned as follows: $|a_2^H| = 0.31$ G and $|a_4^H| = 0.14$ G. These two assignments could be reversed; however, we favor this assignment because

Table 5
EPR parameters 5,8-quinolinesemiquinone [a]

	pH	*g*	a_2^H	a_3^H	a_4^H	a_6^H	a_7^H	a_1^N
50% DMSO [b]	7.4	2.00451	0.31	0.76	0.14	3.04	3.04	0.01
MeOH [b]	7.4	2.00451	0.30	0.78	0.15	3.04	3.04	0.0
CTAB [b]	10.0	2.00473	0.25	0.87	0.0	3.30	2.73	0.0
INDO			−0.44	−0.54	0.21	−1.43	−1.57	−0.02

[a] Hyperfine coupling constants in Gauss, ±0.02 G; *g*-value ±0.00005. Experimental hyperfine coupling constants are absolute values.
[b] Phosphate buffer.



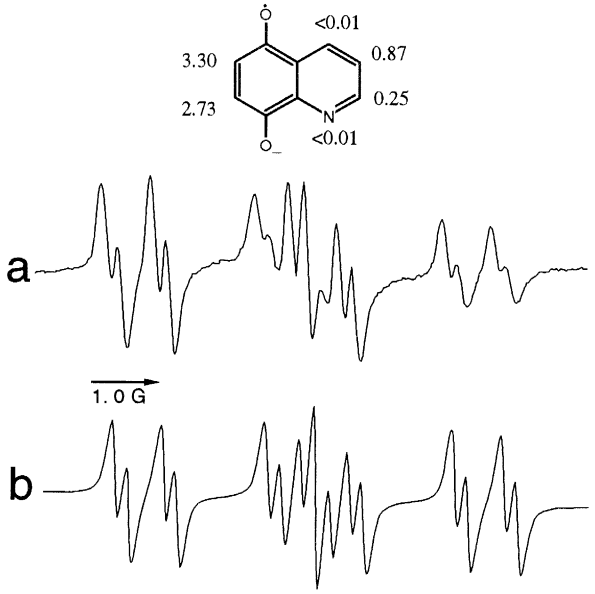


Fig. 9. (a) EPR spectrum of 5,8-quinolinesemiquinone in CTAB/phosphate buffer (pH 10.0) at 25°C. Modulation amplitude, 0.078 G. (b) Computer simulation using the parameters in the inset and in Table 5 with a linewidth of 0.18 G.

it will give a ratio of $|a_4^H/a_3^H| = 0.19$ that is near the corresponding ratio found in $SQ^{\cdot}$ of 0.18. The spectra are independent of the pH from pH 7.40 to 12.0. Above pH 12.0, the spectrum changes from that shown in Fig. 8 to an unknown radical. The radical stability increased with pH from pH 7.40 to pH 10.0. The structure of 5,8-quinolinesemiquinone shows only small changes when prepared in methanol, Table 5. As expected in deuterated solvents, no changes were observed in the EPR spectrum of the 5,8-quinolinesemiquinone radical.

As in the case for $SQ^{\cdot}$, the spectrum of 5,8-quinolinesemiquinone changes significantly when associated with the cationic micelle CTAB as is shown in Fig. 9 (see the discussion below comparing the spectra of the two radicals). The results of INDO calculations are given in the final row of Table 5. Interestingly, these calculations indicate a small, negative spin density on carbon 4 in analogy with $SQ^{\cdot}$.

4. Discussion

Preparation and structural characterization of streptonigrin semiquinone in a series of well-defined experimental conditions provides the first detailed study of this redox intermediate. Specifically, this semiquinone radical has been implicated to be involved in bioactivation and subsequent catalytic single-strand DNA scission promoted by streptonigrin. Closely related aminoquinone antibiotics such as lavendamycin and streptonigrone may also form analogous free-radical intermediates that are likely to be involved in their mechanism of action.

The use of electron paramagnetic resonance spectroscopy to determine the spin distribution and characterize the structural changes of $SQ^{\cdot}$ in response to changing the solvent, varying the temperature, the pH of the medium and its viscosity yielded important information regarding the (1) spin densities of the unpaired electron at the various atoms, (2) motion of the amino group with respect to the plane defined by the quinolinesemiquinone ring, (3) importance of the hydrogen-bonding to the solvent by the $-NH_2$ substituent and its impact on the ability of the amino group to participate in delocalization of the unpaired electron, and (4) conformational change at the AB vs CD ring junction of the antibiotic induced by cationic solvation of the negatively charged semiquinone. Using a theoretical model-compound for molecular orbital calculations and the unsubsituted 5,8-quinolinesemiquinone as an experimental reference radical allowed us to provide further support for both the structural assignments of the coupling constants (spin densities) as well as the observed solvent dependent behavior of $SQ^{\cdot}$.

Reduction of streptonigrin by either sodium borohydride or the synthetic NADH analogue *N*-benzyldihydronicotinamide afforded the same semiquinone radical, reaction 1. The latter has been widely used in nonenzymic dihydronicotinamide reductions as an NADH model since its chemical reactivity is quite comparable to that of the natrually occurring coenzyme [49]. In addition, the fact that both reducing agents yielded the same product shows that sodium borohydride reduced only the quinone function without reacting with any other group in the molecule.

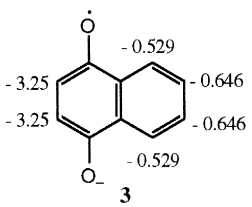
Reduction of streptonigrin by $NaBH_4$ to give $SQ^{\cdot}$ is likely to proceed through two-electron reduction followed by one-electron air oxidation which is consistent with borohydride chemistry. Along these lines, we observed that a large excess of $NaBH_4$ changes the dark brown color of streptonigrin solution to

yellow (formation of dihydroquinone dianion) which turns to dark green only on extended exposure to air yielding the semiquinone as characterized by EPR.

In all solvent systems and under all experimental conditions, significant hyperfine coupling constants were only found for nuclei in the quinolinequinone moiety of the antibiotic indicating that the unpaired electron is delocalized only on that portion of the molecule. The largest coupling constant, contributed by the proton at carbon 3, is in the range $|a_3^H| = 1.72–1.78$ G in various solvents and in neutral or anionic micelles depending on the solvent and the temperature. In cationic micelles $|a_3^H| = 1.54–1.60$ G. Using the McConnell relation [42] $a_3^H = Q\rho_3$ where ρ_3 is the π-electron spin density at carbon 3 and the constant Q is taken to be -25 G, this spin density is about 7.0×10^{-2} in mixed solvents and neutral and anionic micelles and $\rho_3 = 6.2 \times 10^{-2}$ in the cationic micelles. The INDO calculation yields $a_3^H = -1.1$ G corresponding to $\rho_3 = 6.2 \cdot 10^{-2}$ in reasonable agreement with experiment and indicating that the sign of a_3^H is negative, yielding a positive π-electron spin density at carbon 3.

Rather small coupling constants are found for the heterocyclic nitrogen $a_1^N = 0.42–0.50$ G in various solvents and in uncharged or anionic micelles depending on the solvent and the temperature while in cationic micelles, $a_1^N = 0.33–0.36$ G. INDO calculations (Table 1) predict even a smaller coupling.

The coupling constants are small for the other heterocyclic proton attached at carbon 4 for all the solvent systems. INDO calculations predict that a_4^H is positive, corresponding to a negative spin density at carbon 4. Likewise a negative spin density is predicted at carbon 4 in the unsubstituted 5,8-quinolinesemiquinone, Table 5. Kirste [50] studied the naphthosemiquinones **3** and **4**



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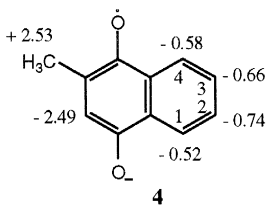

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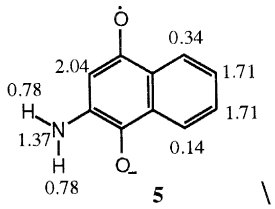
using ENDOR and determined the signs of the hyperfine coupling constants using TRIPLE. The negative coupling constants for hydrogens bonded to carbons 1–4 (using a numbering system comparable with that of **1**) shows that the spin density on these carbons is positive, typical of these types of π-radicals. These same radicals had been studied [36] earlier using EPR with similar results. Comparing radicals **3** and **4** shows that the methyl group has a only a small effect upon the spin density at carbons 1–4. Comparing radical **3** with 5,8-quinolinesemiquinone shows that the presence of the pyridine nitrogen atom (1) has very little effect on the spin density in the quinone ring since the proton coupling constants are -3.25 G versus -3.04 G respectively, and (2) produces a negative spin density at carbon 4. The negative spin density was also inferred from INDO calculations on $SQ^{\cdot}$.

The substituted naphthosemiquinone **5** was studied [36] by EPR in 50% ethanol by volume where the indicated coupling constants are absolute values.



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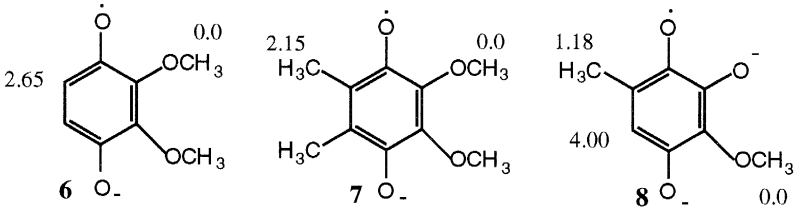
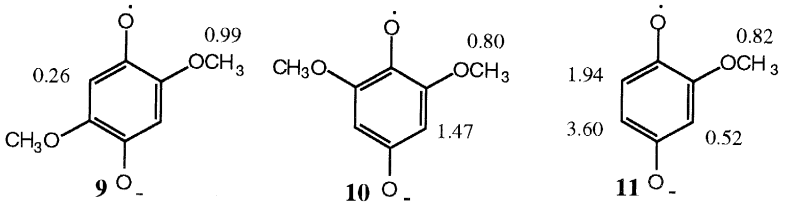
The assignments of the coupling constants to protons at positions 1–4 were not provided in the original work [36] and are tentatively suggested by us by analogy with $SQ^{\cdot}$. Important to this work is the fact that the amino hydrogen and nitrogen couplings are quite similar to those of $SQ^{\cdot}$ in methanol, so the structure of the amino group in radical **5** and in $SQ^{\cdot}$ are similar. In general, amino group coupling constants show a profound variation [30,51]. Amino proton couplings about one-half of those of the nitrogen coupling are sometimes observed [30,51,52] but other ratios are also observed [52–55].

The lack of significant methoxy couplings in $SQ^{\cdot}$ is not surprising as in related radicals these are found to vary with the nature of the ring substituents [30,32–34]. In dilute aqueous sodium hydroxide solution the coupling constants of the methoxy protons have been found to be negligible for the series **6**–**8** [33]

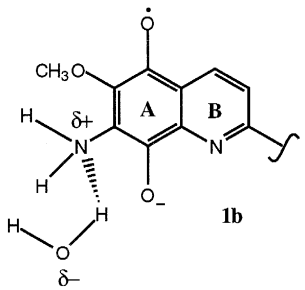


However, the coupling constants of the methoxy protons are significant in the series **9**–**11** in water [34].



Thus the literature results [33,34] for para-semiquinones seem to suggest that the methoxy couplings are negligible if the methoxy group is located next to a second electron donating group; however, the methoxy couplings become significant in the absence of nearby electron supplying groups. In $SQ^{\cdot}$, the methoxy substituent is next to the electron donating amino group, thus its small coupling constant fits into the pattern well.

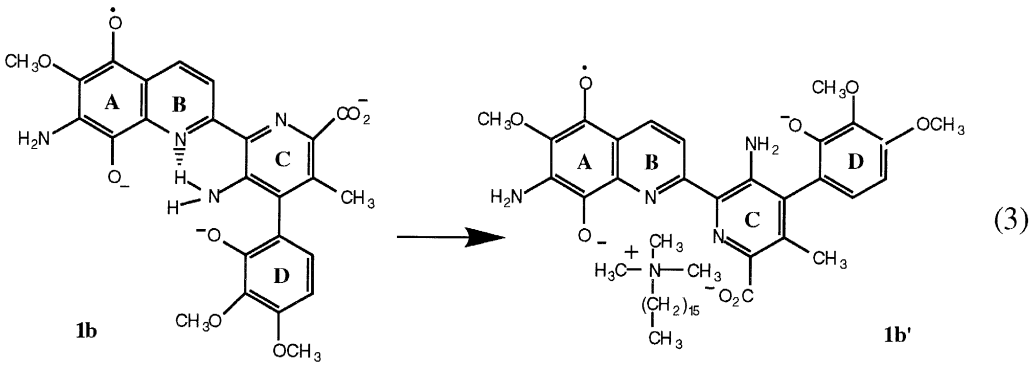
Despite the fact that the appearance of the spectrum of streptonigrin semiquinone varies significantly with both the solvent and temperature, most of the changes may be attributed to coupling constants associated with the amino group of ring A. Since the spin density on ring B varies very little from solvent to solvent and with temperature, no significant reorganization of the electronic wavefunction occurs. Thus, variation in the coupling constants of the protons and the nitrogen of the amino group may best be attributed to changes in the average spatial arrangement of this group. The hyperfine coupling to the amino protons is largely due to hyperconjugation which varies with orientation of the protons. Clearly free rotation of the amino group is ruled out in those cases in which the two protons show inequivalent couplings; nevertheless, rapid motion is implied by the fact that the linewidths are narrow even at some temperatures when the inequivalence persists. On average, the two protons must be positioned asymmetrically with respect to the semiquinone plane and could execute hindered torsional motion. A clue as to the source of hindrance is provided by data presented in Table 3. In the mixed solvents and neutral and anionic micelles, the inequivalence increases monotonically with the nitrogen coupling constant. This observation could be explained by hydrogen bonding between the solvent and the amino nitrogen as follows:



The same hydrogen bond would increase the hindrance to rotational motion and increase the electron withdrawing effect of the nitrogen, favoring the delocalization of the unpaired electron onto the nitrogen.

The structure of the streptonigrin semiquinone is remarkably unperturbed when the antibiotic is associated with membrane mimetic micelles whether they are negatively charged or neutral. Using $|a_3^H|$ as a convenient measure of the spin density in the pyri-

dine ring, this spin density varies by less than 1% in these solvents. The same observation holds for 5,8-quinolinesemiquinone, where a_3^H varies by less than 2%. In contrast, at the surface of cationic micelles, however, $|a_3^H|$ is *reduced* by a significant 10% for streptonigrin and is *increased* by about 12% for 5,8-quinolinesemiquinone. This suggests that the net positive charge at the surface of cationic micelles interacts with these negatively charged radicals in a manner that rearranges the spin density in ring **B**. Since the effects of the charge are in opposite directions in 5-8-quinolinesemiquinone and $SQ^{\cdot}$ respectively, it is reasonable to assume that the secondary **C** and **D** rings in streptonigrin play a role in this rearrangement. A plausible explanation emerges if we assume that the cationic micelles tend to stabilize the conformation of streptonigrin semiquinone **1b′** shown in **1b** → **1b′** [4].



(3)

The conformational change described in **1b** → **1b′** would remove the influence of the amino group in ring **C** on the spin density in ring **B**. The hydrogen bond between the amino group of ring **C** and the quinoline nitrogen in **B** is expected to increase the spin density on the latter thereby increasing the spin density on ring **B**. The conformational change proposed would be favored by the charge–charge interaction between the negatively charged oxygen and the positively charged micellar surface of CTAB. At the same time, the absence of the H-bond between the quinoline nitrogen and the pyridine amino group is expected to decrease the quinoline nitrogen coupling constant. A competing effect is due to the direct effect of a positive charge near the pyridine nitrogen which is expected to increase the spin density in ring **B**. This explanation implying conformational change induced by the positive charge is consistent with observations reported on the complexation of streptonigrin with divalent cations [56]. Upon association of $SQ^{\cdot}$ with a cationic micelle, two competing influences are expected: the positive charge on the micellar surface would increase a_3^H while the loss of the hydrogen bond to the amino group from ring **C** would decrease $|a_3^H|$. A dominance of the latter influence explains the overall decrease in $|a_3^H|$ in $SQ^{\cdot}$.

For 5,8-quinolinesemiquinone, due to the absence of ring **C**, only the charge effect would be operative leading to the increased value of $|a_3^H|$. This hypothesis further predicts that awould be reduced for 5,8-quinolinesemiquinone in the cationic micellar environment which is exactly what has been observed.

Additional support for this interpretation comes from studying the dependence of the spectra on the viscosity of the medium, Table 4. The fact that the rotational correlation time deviates from the Stokes–Einstein equation, Eq. (2), for solvent mixtures of moderate to high viscosities supports involvement of a specific mechanism, such as hydrogen bonding, in addition to the nonspecific viscous drag mechanism. Although the hydrogen bonding mechanism is a vi-

[4] Of the several hydrogen bonds that are likely to be significant, only the hydrogen bond between the amino group of ring C and the quinoline nitrogen is indicated for clarity.

able working hypothesis to describe the behavior of the amino group's coupling constants, further work is necessary to confirm this hypothesis. Along these lines, studying the semiquinone radical in protic versus dipolar aprotic media should provide useful information to resolve the problem.

Preparation and EPR characterization of streptonigrin semiquinone opens an exciting new direction for investigation of the chemistry and mechanism of action of this potent antibiotic. Significantly, the semiquinone radical appears to be sufficiently stable to enable one to begin studies aimed at the elucidation of its physical and chemical properties. As streptonigrin semiquinone has been suggested to function as a key intermediate in the antitumor action in the antibiotic, a better understanding of its redox chemistry with particular emphasis on studies involving the catalytic role of its metal ion-cofactors should provide important insight toward elucidation of the chemical basis of its biological mechanism of action.

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