trans-1-methyl-1,2-cyclohexanediol, 19534-08-8; 1-phenyl-1,2-ethanediol, 93-56-1; 1,2-hexanediol, 6920-22-5; 2,3-butanediol, 513-85-9; 1,2-propanediol, 57-55-6; 3-methyl-2,3-pentanediol, 63521-37-9; glutaric acid, 110-94-1; adipic acid, 124-04-9; pimelic acid, 111-16-0; 6-oxoheptanoic acid, 3128-07-2; benzoic acid, 65-85-0; valeric acid, 109-52-4; acetic acid, 64-19-7; 2-butanone, 78-93-3; phenacyl alcohol, 582-24-1.

The Half-Wave Potentials of 8-Substituted 5-Deazaflavins. Polarographic Determination of the Dissociation Constants of Some 8-Substituted 5-Deazaflavosemiquinones

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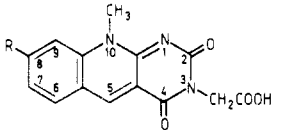
Received October 28, 1985

5-Deazaflavin has been applied as a photocatalyst in the reduction of a wide variety of biological redox systems.[1,2] Its application, however, has serious drawbacks since continuous UV irradiation is required to drive the process and the energy of the required wavelength (300–400 nm) destroys the protein with time. To avoid photodestruction of the protein we have synthesized a number of 5-deazaflavins **1a–g**, which feature a chromophoric group at



1	R
a	Cl
b	NO_2
c	p-NO_2-C_6H_4
d	$(CH_3)_2N$
e	NH_2
f	p-NH_2-C_6H_4
g	p-$(CH_3)_2N$-C_6H_4-N=N

the C(8) position of the 5-deazaisoalloxazine skeleton, causing the absorption maximum to undergo a red shift, and a carboxymethyl group at the N(3) position increasing the solubility of 5-deazaflavins in aqueous media, in which photoreduction of redox enzymes is carried out.[3,4]

Because 8-hydroxy-5-deazaflavin has been found in nature as part of F-420, i.e., the coenzyme of methane-producing bacteria,[5,6] several 8-substituted 5-deazaflavins have been synthesized as model compounds. Their UV spectroscopic data and redox potentials have been examined and found to be very sensitive to the electronic influence of the substituents.[7,8]

Table I. Comparison of Half-Wave Potentials of 5-Deazaflavins 1b and 1e at Different pH Values (in mV vs. NHE)

	pH		
compd	1.90	7.00	9.00
1b	160	−165	−260
	−435	−835	−840
	−710	−1005	−1070
1e	−715	−1000	−1060

Table II. Half-Wave Potentials ($E_{1/2}$) at Different pH Values, Standard Potentials (E_0), and pK_1 and pK_2 Values of 5-Deazaflavins

compd	pH	$E_{1/2}$,[a] mV vs. NHE	E_0,[a] mV vs. NHE	pK_1	pK_2
1a	1.90	−510	−395	1.0 ± 0.2	5.9 ± 0.2
	7.00	−740			
	9.00	−745			
1d	1.90	−775	−615	2.7 ± 0.3	9.3 ± 0.8
	7.00	−1030			
	9.00	−1135			
1e	1.90	−715	−581	2.2 ± 0.2	8.2 ± 0.3
	7.00	−1000			
	9.00	−1060			
1f	1.34	−635	−513	2.0 ± 0.2	7.5 ± 0.2
	6.22	−880			
	7.00	−915			
	8.14	−950			
	9.65	−960			

[a] ±10 mV.

The present report is concerned with the redox properties of a new set of 8-substituted 5-deazaflavins, i.e., 8-chloro- (**1a**), 8-nitro- (**1b**), 8-*p*-(nitrophenyl)- (**1c**), 8-(dimethylamino)- (**1d**), 8-amino- (**1e**), 8-(*p*-aminophenyl)- (**1f**), and 8-[(*p*-(dimethylamino)phenyl)azo]-5-deazaflavin (**1g**). The mechanism proposed[2,9,10] for the photoreduction of enzymes with 5-deazaflavin acting as a photocatalyst, implies the formation of the 5-deazaflavosemiquinone radical. Because of the high reactivity and very low redox potential of the radical, it is possible to reduce a wide variety of enzymes with catalytic quantities of 5-deazaflavin. To investigate the effect of the substituent at the C(8) position on the reducing power of the radical, we wanted to determine the half-wave potentials ($E_{1/2}$) of our 5-deazaflavins by differential pulse polarography.

In addition, the substituent effects on the dissociation constants of the 5-deazaflavosemiquinones have been determined from these polarographic data.

Results and Discussion

Determination of $E_{1/2}$ for the radical formation step of 5-deazaflavins containing a reducible group at C(8) as in **1b,c,g** is not possible because reduction of the group at C(8) takes place at a less negative potential than reduction of the 5-deazaisoalloxazine skeleton. This is exemplified by comparison of the electrochemical reduction of **1b** and **1e** at different pH values (Table I). As can be seen from Table I, the polarogram of **1b** shows two peaks at less negative potential corresponding to the reduction of the nitro group in two steps, followed by a peak at the same potential as obtained for **1e**. The same reduction sequence

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0022-3263/86/1951-1602$01.50/0

is observed with **1c** and **1g**, respectively, i.e., primarily reduction of the nitro and azo function, respectively, followed by reduction of the 5-deazaisoalloxazine skeleton with the yet reduced substituent at C(8).

$E_{1/2}$ values for compounds **1a,d–f** are obtained directly (Table II). The results show the large effect of the substituent at C(8) on the $E_{1/2}$ value of the 5-deazaflavin/5-deazaflavosemiquinone couple.

The $E_{1/2}$ values shift to more negative values with increasing electron-donating character of the substituents. A similar effect has been found in studies on the rate of reduction and reoxidation of 8-substituted 5-deazaflavins.[7c] It was also found that the $E_{1/2}$ values are pH dependent (Table II). This can be explained[11] by the electrode reaction represented by eq 1, in which dFlox stands for oxidized 5-deazaflavin and HdF1· for 5-deazaflavosemiquinone, protonated at N(1).

$$\text{dFlox} + \text{H}^+ + \text{le}^- \rightarrow \text{HdF1}\cdot \tag{1}$$

The Nernst relationship of this redox couple is expressed in eq 2.

$$E = E_0 + \frac{RT}{F} \ln \frac{[\text{dFlox}]\,[\text{H}^+]}{[\text{HdF1}\cdot]} \tag{2}$$

Both the oxidized and semiquinone species have a dissociation equilibrium,

$$\text{HdFlox}^+ \overset{K_1}{\rightleftharpoons} \text{H}^+ + \text{dFlox} \qquad \text{HdF1}\cdot \overset{K_2}{\rightleftharpoons} \text{H}^+ + \text{dF1}^-\cdot$$

The mass action expressions are given in eq 3, with K_1 and K_2 being dissociation constants. Activity effects are neglected.

$$K_1 = \frac{[\text{H}^+]\,[\text{dFlox}]}{[\text{HdFlox}^+]} \qquad K_2 = \frac{[\text{H}^+]\,[\text{dF1}^-\cdot]}{[\text{HdF1}\cdot]} \tag{3}$$

According to the dissociation equilibria in eq 3 both the oxidized and semiquinoide substance can be present in the protonated and deprotonated form. This leads to the expressions in eq 4 for the total concentrations of the oxidized and one-electron reduced species,

$$\begin{aligned} [\text{dFlox}]_t &= [\text{dFlox}] + [\text{HdFlox}^+] \\ [\text{HdF1}\cdot]_t &= [\text{HdF1}\cdot] + [\text{dF1}^-\cdot] \end{aligned} \tag{4}$$

Values of the concentrations [dFlox] and [HdF1·] in eq 2 are obtained from eq 3 and eq 4,

$$[\text{dFlox}] = \frac{K_1[\text{dFlox}]_t}{K_1 + [\text{H}^+]} \qquad [\text{HdF1}\cdot] = \frac{[\text{HdF1}\cdot]_t[\text{H}^+]}{K_2 + [\text{H}^+]} \tag{5}$$

Substitution of eq 5 into eq 2 leads to eq 6 and 7,

$$E = E_0 + \frac{RT}{F} \ln \frac{[\text{dFlox}]_t}{[\text{HdF1}\cdot]_t} + \frac{RT}{F} \ln \frac{K_1([\text{H}^+] + K_2)}{K_1 + [\text{H}^+]} \tag{6}$$

$$E = E_{1/2} + \frac{RT}{F} \ln \frac{[\text{dFlox}]_t}{[\text{HdF1}\cdot]_t} \qquad E_{1/2} = E_0 + \frac{RT}{F} \ln \frac{K_1([\text{H}^+] + K_2)}{K_1 + [\text{H}^+]} \tag{7}$$

where E_0 in eq 6 is the pH independent standard reduction potential of the redox couple dFlox/HdF1· and $E_{1/2}$ in eq 7 is the half-wave potential measured for a given 5-deazaflavin and is pH dependent. As can be seen from eq 7, $E_{1/2}$ is not only defined by E_0 and [H⁺] but also by the dissociation constants K_1 and K_2. This leads to the conclusion that measurement of $E_{1/2}$ as function of the hydrogen ion concentration provides a method for calculating the dissociation constant K_2. This is exemplified by calculation of K_2 of 5-deazaflavin **1f**. It is easy to understand that eq 7 reduces to eq 8a–c for restricted hydrogen ion concentrations as indicated and with the reasonable assumption that K_1 is much larger than K_2.

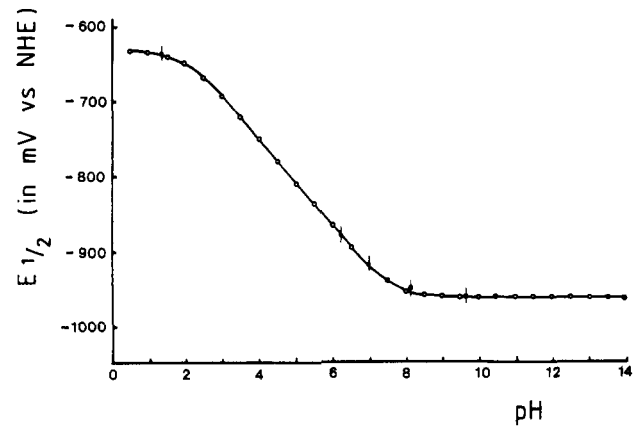

Figure 1. The pH dependence of the half-wave reduction potential ($E_{1/2}$) of 5-deazaflavin **1f**; (○) calculated $E_{1/2}$ values; (●) experimentally determined $E_{1/2}$ values.

$$E_{1/2} = E_0 + \frac{RT}{F} \ln \frac{K_1[\text{H}^+]}{K_1 + [\text{H}^+]} \qquad [\text{H}^+] \simeq K_1 \text{ or } [\text{H}^+] > K_1 \tag{8a}$$

$$E_{1/2} = E_0 + \frac{RT}{F} \ln [\text{H}^+] \qquad K_1 \gg [\text{H}^+] \gg K_2 \tag{8b}$$

$$E_{1/2} = E_0 + \frac{RT}{F} \ln ([\text{H}^+] + K_2) \qquad [\text{H}^+] \simeq K_2 \text{ or } [\text{H}^+] < K_2 \tag{8c}$$

From measurements of $E_{1/2}$ at a pH value below pK_1 (the values of pK_1 have been determined spectrophotometrically) and use of eq 8a, a value for E_0 of −512 mV has been calculated for 5-deazaflavin **1f**. To calculate K_2, $E_{1/2}$ is measured at such a pH value that eq 8c can be used. However, as K_2 is unknown, it is difficult to choose the appropriate pH beforehand. By trial and error we have found that in case of **1f** the $E_{1/2}$ value obtained at pH 9.65 differs distinctly from the value calculated according to eq 8b. This is certainly caused by the participation of the dissociation of the 5-deazaflavosemiquinone in the overall electrode reaction, eq 8c being valid as a result. Applying now eq 8c we have calculated a pK_2 value of 7.6 for **1f**.

Using the measured and calculated values for pK_1, E_0, and pK_2 we have plotted calculated $E_{1/2}$ values against pH according to eq 7 (Figure 1).

As can be seen from Figure 1, the experimentally determined $E_{1/2}$ values are well in line with the calculated $E_{1/2}$ vs. pH plot showing the validity of our theoretical approach. A more accurate calculation of pK_2, taking all experimentally determined $E_{1/2}$ values into account, finally leads to a value of 7.5. The same procedure has been applied for the 5-deazaflavins **1a,d,e**. The results are shown in Table II.

Since the pK_2 value of the unsubstituted 5-deazaflavin is reported to be very divergent (pK_2 = 5[9] and pK_2 = 8[12]), we wanted to establish more accurately its value from a possible correlation between the pK_2 values of compounds **1a,d–f** and their respective σ_p values. However, the σ_p value of the *p*-aminophenyl substituent is unknown. As it has been demonstrated[13] that there exists a relationship

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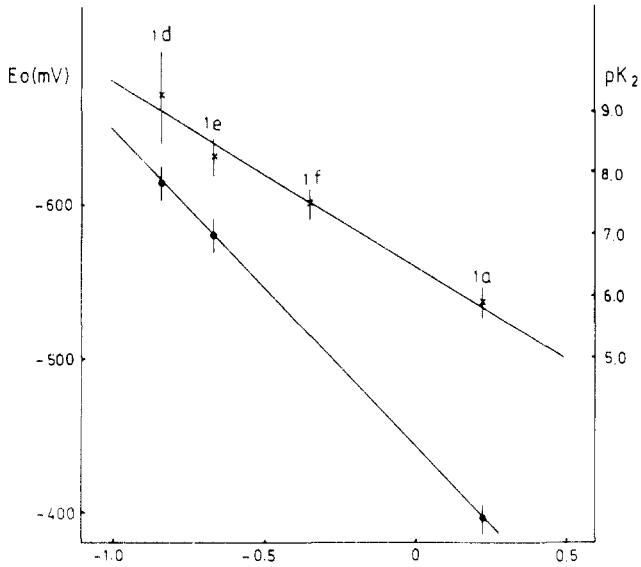

Figure 2. E_0 values of compounds **1a,d,e** vs. σ_p (●); pK_2 values of compounds **1a,d-f** vs. σ_p (×).

between reduction potentials and sigma values in organic systems, we determined the σ_p value of that substituent by the relationship found between the E_0 values of compounds **1a,d,e** and their respective σ_p values[14] (Figure 2).

From this relationship we calculated a σ_p (*p*-aminophenyl) value of −0.34. The correlation of the σ_p values of the four substituents with pK_2 is shown in Figure 2, from which we could derive a pK_2 value for the unsubstituted 5-deazaflavin of 6.5 ± 0.2.

As mentioned in the literature,[12] kinetic $E_{1/2}$ values rather than thermodynamic potentials have been obtained due to dimerization of the respective radicals. Values for thermodynamic $E_{1/2}$ potentials are either equal to or more negative than the respective values for kinetic $E_{1/2}$ potentials, depending on the rates of radical dimerization.

Despite the fact that we have carried out our measurements at rather arbitrary concentrations of **1a-g** we still found a good relationship between $E_{1/2}$ and pH values which points to an apparent lack of a concentration dependence of $E_{1/2}$; i.e., the dimerization rate is low compared to the overall electrode reaction rate.

Comparison of the data in Table II with respect to the substituent at C(8) leads to the conclusion that $E_{1/2}$ potentials shift to more negative values and that pK_1 and pK_2 values increase with increasing electron-donating character of the substituent. The fact that the E_0 and pK_2 values meet the Hammett relation points at the importance of through resonance in the reduction and dissociation process.

Experimental Section

Half-wave potentials for $1e^-$ reduction of 5-deazaflavins have been determined by differential pulse polarography with a Quickstep instrument.[15] A saturated Ag/AgCl electrode was used as reference against the static mercury drop electrode (repeating mode). The following buffers were used: 0.1 M KCl/HCl buffer, pH 1.34 and 1.90; 0.1 M phosphate buffer, pH 6.22 and 7.00; 0.1 M Tris buffer, pH 8.14 and 9.65. Concentrations of 5-deazaflavin solutions were varied between 30 and 100 μM. The temperature was 20 ± 2 °C.

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Acknowledgment. We are indebted to Dr. H. P. van Leeuwen and Dr. H. G. de Jong, Laboratory for Physical and Colloid Chemistry, Agricultural University, Wageningen, for reading the manuscript.

Registry No. **1a**, 98629-96-0; **1d**, 98629-99-3; **1e**, 98630-00-3; 1f, 98630-01-4.

Hybridization of the Lone Pair Electrons in Amines and the Corresponding N-H Bond in Protonated Amines

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Received November 9, 1985

Theoretical studies on amines by ab initio,[1] semiempirical,[2] and more recently, molecular mechanics[3] calculations are well documented. However, to the best of our knowledge, no report on the hybridization of the lone pair electrons in amines has appeared in the literature. We recently reported a localized molecular orbital (LMO) calculation of the hybridization of the lone pair electrons in carbanions.[4] Rehybridization has been found to occur in either direction depending on the structure of the carbanions. For example, the s character of the lone pair orbital of the methyl anion is significantly enhanced over that of the corresponding C-H bonding orbital of the parent methane molecule, while decreases in s character are found in other acyclic anions. Since an amine is isoelectronic with its corresponding carbanion, it would be interesting and desirable to compare the hybridization of the lone pair orbitals in amines with that in carbanions. We now wish to describe an LMO study on the hybridization of the lone pair electrons in amines and, for the purpose of comparison, the hybridization of the N-H bond in protonated amines.

Calculations

The ground-state geometries of the amines and the protonated amines under investigation were fully optimized at the INDO level by using a locally modified CINMIN program.[5] The optimization procedure of this program uses an adapted version of the method due to Rosenbrock.[6] The MO's obtained were transformed into localized orbitals, LMO's, by the technique of Edmiston and Ruedenberg[7] using the ORBLOC subroutine.[8] These LMO's are energy-localized orbitals which minimize the sum of all interorbital repulsions and the sum of the self-energies

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