Electron Spin Resonance Study of Radicals Derived from Simple Amines and Amino Acids

R. Poupko, A. Loewenstein,* and Brian L. Silver

Contribution from the Department of Chemistry, Technion–Israel Institute of Technology, Haifa, Israel. *Received August 15, 1969*

Abstract: Radicals derived from methyl-, ethyl-, dimethyl-, and diethylamines, glycine, and α-alanine were studied by esr spectroscopy. All the radicals were produced by interaction with Ti^{3+}–H_2O_2 in a flow system at pH 9–12. The spectra of the radicals derived from alkylamines were interpreted in terms of nitroxides. Dimethyl- and diethylamines give two different nitroxides depending on the flow rate. Methyl- and ethylamines give radicals apparently formed by reaction of the initially formed radical with an unreacted molecule. The spectra of the amino acid radicals show temperature-dependent proton hyperfine splittings from the amine hydrogens. The experimental results are interpreted in terms of noncoplanarity of the NH_2 group with the nodal plane of the π system. Theoretical estimates are made of the splitting constants in the amino acid radicals.

A considerable number of radicals derived from the interaction of organic molecules with Ti^{3+} and H_2O_2 in a flow system have been studied by the electron spin resonance (esr) technique.[1-5] The spectra can usually be interpreted in terms of radicals formed by hydrogen abstraction or by the addition of an OH radical to a double bond.[4] In alcohols hydrogen abstraction is usually in the α position to the hydroxyl group while in acids and acidic solutions of amines the site of abstraction is generally far removed from the functional group. Also in amines or acids the yield of radical is much lower than in alcohols. This has been attributed to deactivation induced by the functional group which renders the molecule less susceptible to electrophilic attack by the OH radical.[1] The type and number of radicals produced are often a function of the experimental conditions such as pH, concentration of the reagents, flow rate, temperature, etc.[1,3,6-9]

In this communication we report a detailed study of radicals derived from simple amines and amino acids. The materials studied were methyl-, ethyl-, dimethyl-, and diethylamine, glycine, and α-alanine. Since we usually worked in the presence of *N,N'*-ethylenediaminetetracetic acid (EDTA), a brief discussion of the spectrum of the radical derived from EDTA is also added. All compounds were studied in basic media (pH 9–12). Some of these systems have been studied previously with various degrees of completeness.[2,3,5]

The interest in these systems is twofold. (a) The correct identification of these relatively simple radicals should lead to a better understanding of more complicated systems and in particular the radicals derived from amino acids and peptides. The results could also further our knowledge of oxidative processes of amines induced by irradiation and other agents. (b) The interpretation of the hyperfine splitting constants (in particular those due to ^{14}N) is of considerable theoretical interest. In contrast to the ^{13}C polarization constants (Q^{C}_{ij}) which are relatively well understood, the ^{14}N parameters (Q^{N}_{ij}) are still the subject of controversy. In particular, the magnitude and even the sign of the polarization of the CN bond by the electron on the neighboring carbon atom are not settled.[10] The reason is that in the expression for the hyperfine splitting[11]

$$a^{N} = (S_N + \sum_i Q^{N}_{NX_i})\rho_N{}^{\pi} + \sum_i Q^{N}_{X_iN}\rho_{X_i}{}^{\pi}$$

the second term is usually small because the spin density on the neighboring atom is small. Our original intention was to obtain radicals from amines by hydrogen abstraction in the α position to the NH_2 group (similar to those obtained from alcohols) and thereby increase the spin density on the adjacent carbon and consequently the second term in the expression for a^N. This, however, was achieved only for the amino acids and EDTA. A detailed theoretical investigation concerned with the calculation of the carbon and nitrogen hyperfine splittings is given in the accompanying paper.[12]

We have chosen to work in basic solutions because it is reasonable to assume that in this medium proton abstraction is more likely to occur close to the functional group. Furthermore, the reactivity of the amines and amino acids toward the OH radical is much greater at high pH values.[13] In acidic media strong deactivation toward attack by the OH radical has been observed and attributed to the influence of the ionized amine group. This may also be the reason that no radicals from methylamine in either acidic or basic media have been observed previously.[2,3,14]

Experimental Section

Materials. All materials were commercially available and used without further purification. $TiCl_3$ solution was synthesized by the

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Table I. Summary of Experimental Conditions

Substrate	$[Ti^{3+}]$, M	$[H_2O_2]$, M	[Substrate],[b] M	pH[d]	Flow rate, ml/min	Temp, °C
CH_3NH_2	5×10^{-3}	$(3-5) \times 10^{-2}$	$(5-20) \times 10^{-2}$	10–12	50–300	25
$C_2H_5NH_2$	5×10^{-3}	5×10^{-2}	7×10^{-2}	10–12	50–300	25
$(CH_3)_2NH$	5×10^{-3}	5×10^{-2}	5×10^{-2}	10–12	100–500	25
$(C_2H_5)_2NH$	5×10^{-3}	5×10^{-2}	$(3-4) \times 10^{-2}$	10–12	40–500	25
$NH_2CH_2COO^-$	6×10^{-3}	$(2-6) \times 10^{-2}$ [a]	3×10^{-1}	9–11	200–450	3–55
$NH_3^+CH_2COO^-$	9×10^{-3}	4×10^{-2}	5×10^{-1}	3–5	450	15–35
$NH_2CH(CH_3)COO^-$	6×10^{-3}	$(2-6) \times 10^{-2}$ [a]	5×10^{-1} [c]	9–11	300–450	1.5–50
EDTA	5×10^{-3}	$(3-6) \times 10^{-2}$	2×10^{-2}	8–12	80–100	25

[a] At high temperatures lower H_2O_2 concentrations were used. [b] Compounds were dissolved in both H_2O_2 and Ti^{3+} solutions. In several cases the compound was dissolved only in the Ti^{3+} solution, with identical results. [c] Compound dissolved only in the Ti^{3+} solution because of reaction with H_2O_2. [d] The pH values were measured in both Ti^{3+} and H_2O_2 solutions before mixing using a Metrohm Type E184C pH meter and combined glass–calomel electrodes. The pH was adjusted by the addition of concentrated KOH or NH_4OH solutions.

electrochemical reduction of $TiCl_4$.[15] Distilled water was used in the preparation of all solutions. Details of the composition of the solutions and the flow rates are given in Table I.

All solutions at basic pH contained EDTA in an equal concentration to that of the Ti^{3+}. The spectrum of the EDTA radical was observed only when EDTA was in excess of this concentration. To verify that the radicals obtained from the other amines are not affected by the presence of the EDTA, we ran several spectra in the absence of EDTA and obtained identical results. The spectra, however, were of much lower intensity. In such cases both the substrate and the base were dissolved in the H_2O_2 solution. During mixing the titanium did not visibly precipitate, presumably because the rate of its oxidation is very fast.

Spectrometer. Spectra were recorded on a Varian V4502 epr spectrometer operating at 9.5 GHz with 100-kHz field modulation. Solutions were mixed in a Varian V4549 mixing chamber. The temperature was controlled to within ±0.2° by thermostating the reservoirs containing the reactants. The field was calibrated against the splitting in Fremy's salt (13.05 G).

Results

CH_3NH_2 and $C_2H_5NH_2$. Experimental and computer-simulated spectra for radicals obtained from CH_3NH_2 and $C_2H_5NH_2$ are given in Figures 1 and 2.

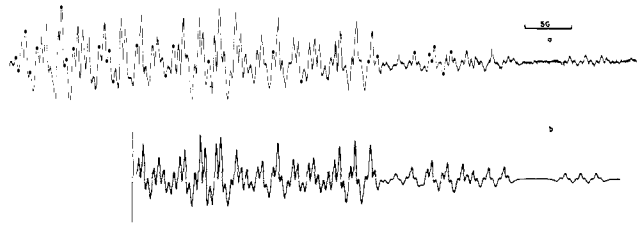

Figure 1. (a) The second-derivative spectrum of the radical derived from CH_3NH_2. The spectrum is symmetrical and only part of the low-field lines are shown. (b) Computer simulation of the high-field half of the spectrum using parameters given in Table II. The line width taken was 0.9 G. The vertical line indicates the center of the spectrum.

The spectrum of the radical derived from CH_3NH_2 is given as the second derivative because of its complexity. The parameters used for the simulated spectra are given in Table II. It was generally observed that an increase in the amine concentration beyond a certain value *decreases* the intensity of the observed spectrum.

$(CH_3)_2NH$ and $(C_2H_5)_2NH$. Two radicals are formed from each compound, depending on the flow rate. Radical A is formed at fast flow rates and B at slow flow rates. The spectra are shown in Figures 3 and 4 and their interpretation is given in Table II. The relation between substrate concentration and intensity mentioned above for methyl- and ethylamine was not observed here.

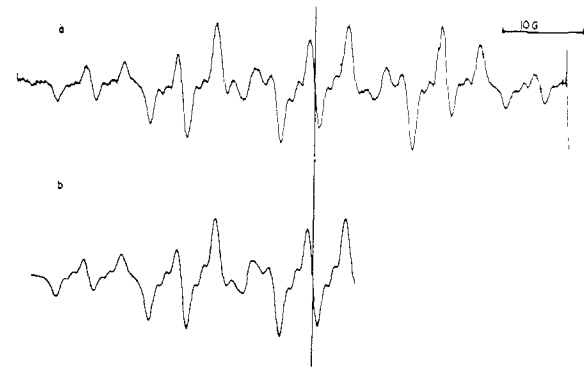

Figure 2. (a) The first-derivative spectrum of the radical derived from $C_2H_5NH_2$. The spectrum is symmetrical and the vertical line indicates its center. (b) Computer simulation (line width, 1.3 G) of the low-field half of the spectrum using parameters given in Table II.

Amino Acids. The spectra of the radicals derived from glycine and alanine in basic media are shown in Figures 5 and 6. The spectral parameters are given in Table II. The splittings due to the NH_2 hydrogens change with temperature the variations being shown in Figure 7.

EDTA. The spectrum (second derivative) is given in Figure 8 and the spectral parameters are in Table II.

Discussion

CH_3NH_2. Irradiation of solid CH_3NH_2 gives the CH_3NH radical with seven equal splittings of 32 G.[16] The $CH_2NH_3^+$ radical has also been observed in the solid following irradiation of methylammonium alum.[17] The CH_3NH radical might be a precursor of the $CH_3(NO)CH_2NH_2$ radical which we observed. The identification of the radical is based on comparison of the computer-simulated and experimental spectra. Additional support for the identification is obtained by a comparison of the derived hyperfine splittings with those obtained in similar radicals such as $(CH_3)_2NO$[18] and $(C_2H_5)_2NO$.[3,18,19]

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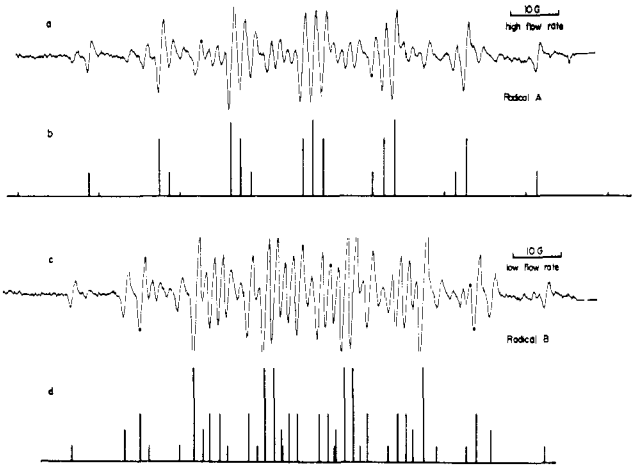

Figure 3. Experimental (first derivative) and calculated spectra for radicals derived from $(CH_3)_2NH$ at high and low flow rates. Identification of the radicals and their parameters are given in Table II. The spectra of A and B contain residual lines due to B and A, respectively.

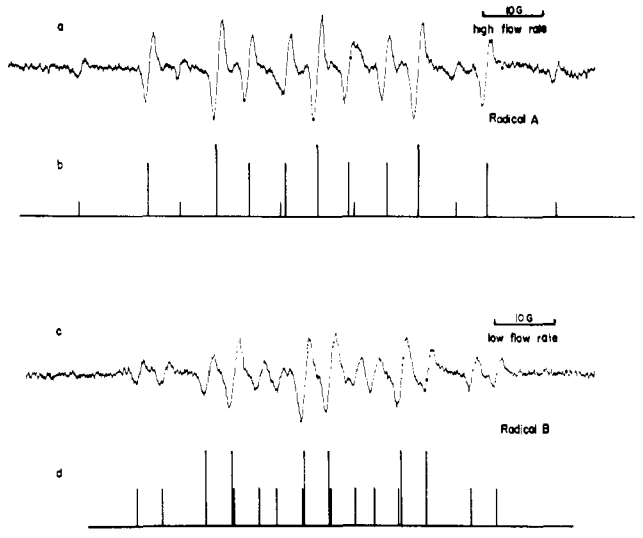

Figure 4. Same as Figure 3 for the radicals derived from $(C_2H_5)_2NH$.

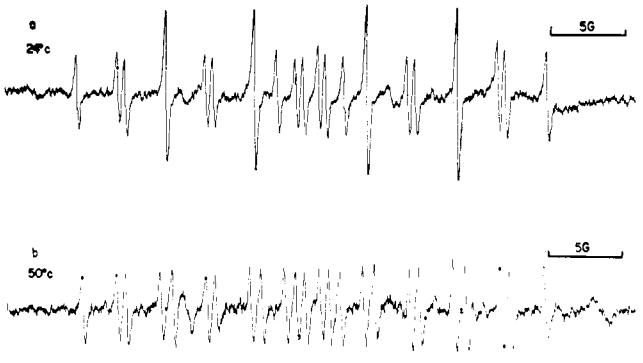

Figure 5. The first-derivative spectrum of the radical derived from glycine in basic media.

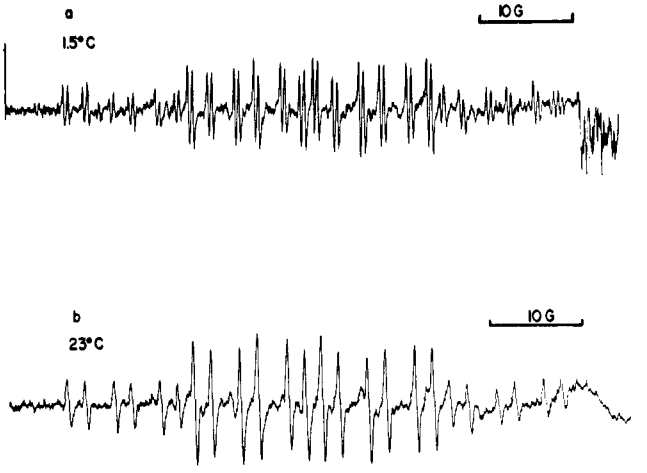

Figure 6. Same as Figure 5 for the radical derived from α-alanine. The broad line at the right-hand side at 23° is due to Ti-EDTA complex.

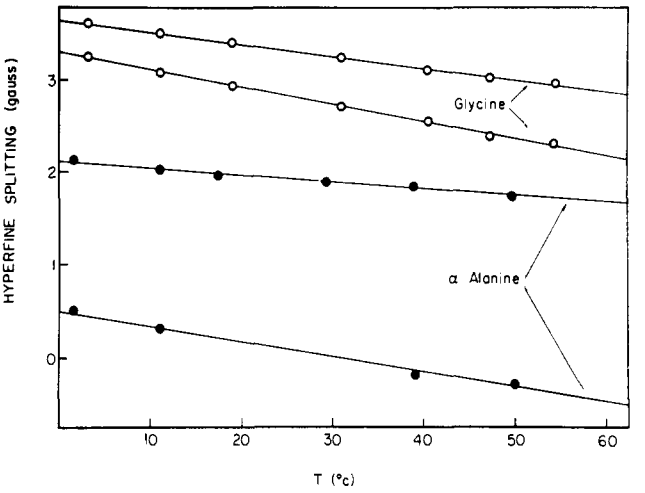

Figure 7. Hyperfine splittings of the amine hydrogens in radicals derived from glycine and alanine in basic media as a function of the temperature.

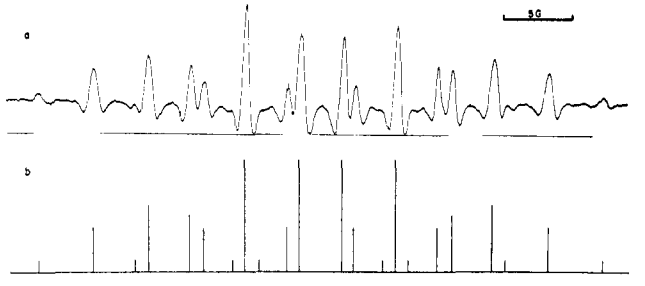

Figure 8. Experimental (a) and calculated (b) second-derivative spectra of the radical derived from EDTA. The parameters are given in Table II.

It is uncertain whether the primary radical is formed by the breaking of a CH or NH bond. The production of either CH_2NH_2 or CH_3NH could eventually lead to the observed radical *via* interaction between two radicals or a radical and a unreacted molecule. The fact that the production of this radical is not proportional to the amine concentration is not understood.

$C_2H_5NH_2$. The assignment of the hyperfine constants is again based on computer-simulated spectra and the comparison of the magnitude of the hyperfine splitting with similar radicals such as $(CH_3)_2CH(NO)$-$CH(CH_3)_2$ and $(C_2H_5)NO$.[18a] The structure of the radical shows that proton abstraction does not occur at the β carbon as it does in acidic solution.[2] A

Table II. Hyperfine Parameters

Compound	Relative intensities	Hyperfine splittings, G	Assumed radical
CH_3NH_2	(1:1:1) (1:3:3:1) (1:2:1) (1:1:1) (1:2:1)	$a^N_{NO} = 16.4$ $a^H_{CH_3} = 14.1$ $a^H_{CH_2} = 9.7$ $a^N_{NH_2} = 1.6$ $a^H_{NH_2} = 0.45$	$CH_3N(O)—CH_2NH_2$
$C_2H_5NH_2$	(1:1:1) (1:2:1) (1:1) (1:1:1) (1:3:3:1) and/or (1:2:1)	$a^N_{NO} = 16.4$ $a^H_{CH_2} = 11.6$ $a^H_{CH} = 4.6$ $a^N_{NH_2} = 1.3$ $a^H_{CH_3} = 0.4$ and/or $a^H_{NH_2} = 0.4$	$CH_3CH_2N(O)—CH(CH_3)NH_2$
$(CH_3)_2NH$, A	(1:1:1) (1:6:15:20:15:6:1)	$a^N_{NO} = 16.9$ $a^H_{CH_3} = 14.8$	$CH_3—N(O)—CH_3$
B	(1:1:1) (1:3:3:1) (1:2:1)	$a^N_{NO} = 16.5$ $a^H_{CH_3} = 14.6$ $a^H_{CH_2} = 11.3$	$CH_3—N(O)—CH_2X$ (X = OH?)
$(C_2H_5)_2NH$, A	(1:1:1) (1:4:6:4:1)	$a^N_{NO} = 16.9$ $a^H_{CH_2} = 11.6$	$(C_2H_5)_2N—O$
B	(1:1:1) (1:2:1) (1:1)	$a^N_{NO} = 16.3$ $a^H_{CH_2} = 11.7$ $a^H_{CH} = 4.2$	$C_2H_5N(O)—CHXCH_3$ (X = OH?)
$NH_2CH_2COO^-$	(1:1) (1:1:1) (1:1) (1:1)	$a^H_{CH} = 13.8$ $a^N_{NH_2} = 6.1$ $a^H_{NH_2} = 2.9\text{–}3.6^a$ $2.3\text{–}3.2^a$	NH_2CHCOO^-
$NH_2CH(CH_3)COO^-$	(1:3:3:1) (1:1:1) (1:1) (1:1)	$a^H_{CH_3} = 13.9$ $a^N_{NH_2} = 5.1$ $a^H_{NH_2} = 1.7\text{–}2.1^a$ $0\text{–}0.5^a$	$NH_2C(CH_3)COO^-$
EDTA	(1:1) (1:1) (1:4:6:4:1)	$a^H_{CH} = 11.1$ $a^N_{NCH_2} = 7.1$ $a^H_{CH_2} = 4.0$	$—OOC—CH(N(CH_2)_2N(CH_2COO^-)_2)—CH_2—OOC$

[a] See Figure 7 for the temperature variation of the splitting constant.

variety of mechanisms can be suggested to account for the radicals obtained with primary amines, but without a detailed kinetic study it is not possible to determine the reaction mechanism.

$(CH_3)_2NH$ and $(C_2H_5)_2NH$. The formation of two radicals which depend on the flow rate has been noted previously under different conditions by Florin, *et al.*[3] Our assignment of splitting constants for radical A in $(CH_3)_2NH$ is identical with theirs but they did not assign splitting constants to radical B. In $(C_2H_5)_2NH$ both radicals A and B have been identified previously.[3] Our interpretation differs slightly in that we assume that X = OH in B, whereas Florin, *et al.*, propose a negative radical ion instead.

Amino Acids. In amino acids in basic media hydrogen abstraction occurs at a carbon atom adjacent to the amino group. This behavior is similar to that observed in alcohols. In contrast to the simple amines described above, the production of a secondary radical is not observed. This presumably is due to the stabilizing effect of the carboxylate group.

Glycine. A preliminary account of the spectra of the radicals derived from glycine and alanine in basic media has been given previously.[20] The spectrum of glycine in acid media has been given by Taniguchi, *et al.*,[2] and by Armstrong, *et al.*[5] We have repeated their work under slightly different conditions and obtained similar results. The hyperfine constants for the $NH_3^+CHCOO^-$ radical are $a^H_{CH} = 12.2$ G, $a^N_{NH_2} = 6.4$ G, and $a^H_{NH} = 5.4$ G (in comparison, the values obtained by Taniguchi, *et al.*, are 12.4, 6.6, and 5.5 G, respectively). In addition to the spectrum assigned to the glycine radical, there are additional lines, forming an identical pattern with that of the glycine radical, but displaced upfield by 0.7 G from the latter spectrum and having significantly greater line width (see Figure 9).

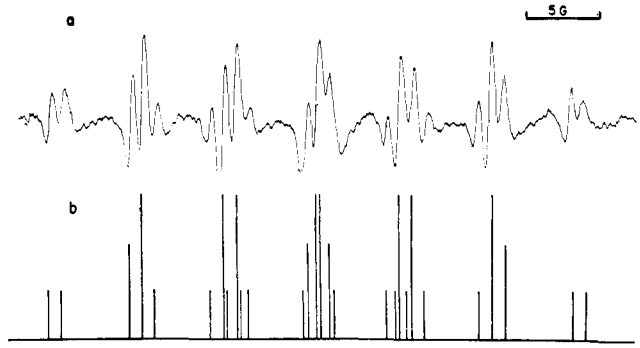

Figure 9. Second-derivative spectrum of the radical derived from glycine at pH ~4. The simulated spectrum shown below is a superposition of the spectra of two identical radicals with slightly different g values. The hyperfine parameters used are those given in the text.

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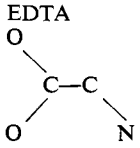
Table III. Calculated Spin Densities

			ρ_C	ρ_N	ρ_{COO^-}
Glycine	Estimated from experiment as described in text	Acidic	0.50	0.35	
		Basic	0.56	0.29	0.15[a]
EDTA			0.46	0.39	
	LCAO-MO-SCF (closed shell)[b]		0.623	0.237	0.140
	VESCF[c]		0.495	0.341	0.164
	LCAO-MO[d]		0.53	0.26	0.21

[a] Estimated. [b] Method given by Forster, ref 27. [c] Method given by Heffernan and Brown, ref 28. [d] See ref 2.

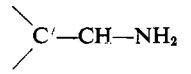
One explanation could be that this spectrum is due to glycine radical complexes with titanium *via* the carboxylate group. It is known that titanium complexes easily with oxygen atoms in organic molecules. In addition if the experiment is carried out in the presence of EDTA the intensity of the total spectrum decreases considerably. Taniguchi, *et al.*, failed to observe this complexity of the spectrum because under their conditions the line width was about 1.5 G. The spectra in acidic and basic media have quite different appearance but probably represent the same radical with a slightly different splitting constant.

The spectrum in basic media shows an interesting feature in that the amine hydrogens are not equivalent and their splitting constants are temperature dependent. This effect may result from some kind of hindered rotation about the C–N bond whose rate is temperature dependent. A similar situation occurs in the CH_2-$CONH_2$ radical[21] where the NH_2 hydrogens are nonequivalent, although no temperature variation studies were made. The proton splitting from the OH group of protonated *p*-benzosemiquinone shows a similar temperature dependence.[22] Fessenden[23] has discussed the temperature variation of β-proton splittings, but for almost freely rotating groups in which no inequivalence occurs.

Alanine. The general behavior in alanine is similar to that observed for glycine. In our preliminary communication[20] we did not report an inequivalence in the splittings of the two NH_2 hydrogens, since at room temperature one of the splittings is in fact zero. It was consequently assumed that one of the NH_2 hydrogens is replaced by an OH group. The smaller proton splitting of the NH_2 group depends linearly on temperature if it is assumed that the sign of the splitting constant changes from positive to negative with rising temperature. The radical derived from alanine in acidic media has been observed by Taniguchi, *et al.*,[2] who found that proton abstraction occurs in the CH_3 group.

EDTA. The radical derived from EDTA has been identified by us[20] and by others.[5,9]

Hyperfine Splitting Constants in Amino Acids. In order to use the measured hyperfine splitting constants of amino acids to derive spin densities it is necessary to know the values of the relevant σ–π polarization parameters. A theoretical estimation has been made[12] of the Q matrix for the π fragment



This fragment occurs in many amino acid radicals and carries almost all the unpaired spin. The method of calculation for the Q matrix lays emphasis upon the orthogonalization of the set of interatomic σ bonds.[24] We now attempt to rationalize the hyperfine constants listed in the Results section in terms of the theoretical Q matrix. The carbon-bonded hydrogen and the nitrogen-bonded hydrogens are treated separately. The theoretical result for the hyperfine constant a^H_{CH} in the fragment shown above is

$$a^H_{CH} = -26.1\rho_C^\pi - 2.6\rho_{CN}^\pi - 0.4\rho_N^\pi + Q^H_{CC'}\rho_{CC'}^\pi + Q^H_{C'C}\rho_{C'}^\pi \quad (1)$$

The symbols in eq 1 are those used by McConnell.[25] (Subsequently the superscript π will be dropped.) In glycine and alanine carbon C′ belongs to a carboxylate group. The last two terms on the right-hand side of (1) are presumably negligible since both the spin density on C′ and the polarization constants $Q^H_{CC'}$ and $Q^H_{C'C'}$ are small. The amount of spin density on the carboxylate group (ρ_{COO^-}) is estimated to be about 0.15. This value is in the range of calculated results obtained by different methods (*cf.* Table III) and agrees with the small spin delocalization observed in the CH_2COO^- radical. We have measured the esr spectrum of this radical at pH 9 and found a^H_{CH} = 20.9 G, which differs only slightly from the value of 23 G observed in the CH_3 radical.[26] Now since $\rho_C = c_C^2$, $\rho_N = c_N^2$, and $\rho_{CN} = c_C c_N$, where the c's are atomic π-orbital coefficients, it follows that

$$\rho_{CN} = -(\rho_C\rho_N)^{1/2} \quad (2)$$

The negative sign follows from the opposite signs of c_N and c_C which come out of the MO calculations. Taking $\rho_{COO^-} = 0.15$, we set $\rho_C + \rho_N = 0.85$. From this value and the measured $a_{CH}{}^H$ (see Table II), ρ_C and ρ_N for glycine and EDTA were evaluated using eq 1. These estimated values together with those obtained from LCAO-MO-SCF calculations[27,28] for a

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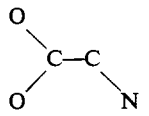
Table IV. Calculated Splitting Constants

Splitting constant		$^-$OOCCHNH$_2$ (basic)	$^-$OOCCHNH$_2$ (acidic)	$^-$OOCC(CH$_3$)NH$_2$	$^-$OOCCH–N(R)–CCH$_2$OO$^-$ (EDTA)
$a^{H_1}_{NH}$	From eq 3a	7.5; 8.1	8.9; 9.5	7.5; 8.1	
	From eq 4a	5.77	6.95	5.77	
	Obsd	2.3–3.2	5.4	1.7–2.1	
$a^{H_2}_{NH}$	From eq 3a	7.5; 8.1	8.9; 9.5	7.5; 8.1	
	From eq 4b	5.82	7.02	5.82	
	Obsd	2.9–3.6	5.4	0–0.5	
$a^{N}_{NH_2}$	From eq 3b	6.1; 5.8	7.6; 7.5	6.1; 5.8	9.0; 8.9
	From eq 4c	2.07	3.32	2.7	4.5
	Obsd	6.1	6.4	5.1	



fragment are given in Table III. Also in Table III are values calculated by Taniguchi, *et al.*[2]

We now turn to an estimate of ρ_C and ρ_N in alanine. The splitting of β hydrogens in a methyl group is given by $a^{H}_{C'-CH_3} = (B_0 + B_2\langle\cos^2\theta\rangle)\rho_C$, where $(B_0 + B_2\langle\cos^2\theta\rangle)$ is usually taken as equal to 25 G for a freely rotating methyl group.[29] Hence ρ_C(alanine) = $^{13.9}/_{25}$ = 0.56, where 13.9 G is the observed CH_3 splitting (*cf.* Table II). If we now assume ρ_{COO^-} in alanine to be equal to that in glycine (0.15) we obtain ρ_N(alanine) = 0.29.

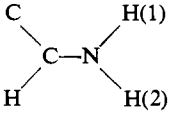
From the spin densities estimated above it is possible to derive values for the hyperfine constants using the relevant Q values. We do this using two sets of values, one derived theoretically by the present authors,[12] and another set given by Smejtck, Hanzl, and Metalova[30] and derived semiempirically from the splitting constants of a number of aromatic amine radicals. These authors use Karplus and Fraenkel's[11] nomenclature. In terms of the nomenclature used in this paper their equations are

$$a^{H}_{NH_2} = Q^{H}_{NH}\rho_N \quad (3a)$$

$$a^{N}_{NH_2} = Q^{N}_{NN}\rho_N + Q^{N}_{CC}\rho_C \quad (3b)$$

where $Q^{H}_{NN} = -26$ or -28 G, $Q^{N}_{NN} = 26$ or 27 G, and $Q^{N}_{CC} = -2.6$ or -3.6 G.

If we turn to the results obtained for the parameters of the fragment



we find the following expressions for the hyperfine splitting constants[12]

$$a^{H_1}_{NH} = -24.3\rho_N - 3.2\rho_{CN} \quad (4a)$$

$$a^{H_2}_{NH} = -24.5\rho_N - 3.2\rho_{CN} \quad (4b)$$

$$a^{N}_{NH_2} = 19.4\rho_N + 1.9\rho_{CN} - 5.0\rho_C \quad (4c)$$

Using the values for ρ given in Table III in eq 3 and 4 we obtain the results shown in Table IV.

It is evident from Table IV that the calculated values for the hyperfine splittings are still in considerable disagreement with the experimental results. The parameters of ref 30 give better agreement than ours[12] between experiment and theory for the ^{14}N splittings, and worse agreement for the proton splittings. If our parameters are accepted, then the fact that $a^{N}_{NH_2}$(obsd) is much larger than $a^{N}_{NH_2}$(calcd) might be explained by a bending of the amine group in relation to the $^-$OOC–C plane. The bending will mix the $2p_z$ orbital with the 2s orbital on the nitrogen. The change in the hyperfine splitting is given by[31]

$$a^{N}_{NH_2}(\theta) = a^{N}_{NH_2}(0) + 550(2\tan^2\theta)\rho_N \quad (5)$$

where 550 G is the value of splitting caused by one electron in a nitrogen 2s orbital[32] and θ is the angle between the $2p_z$ orbital and the perpendicular to the C–COO$^-$ plane. We use the above equation to estimate θ by setting the calculated nitrogen hyperfine splitting equal to $a^{N}_{NH_2}(0)$ and $a^{N}_{NH_2}(\theta)$ to the observed splitting constants. We derive values of θ = 6.5, 5, 5.5, and 4.5°, respectively, for glycine in basic and acid solutions, alanine, and EDTA. The values for θ do not depend strongly on the spin density chosen for the nitrogen atom. The same mechanism will affect the hydrogen splitting constant of the NH_2 group by introducing positive spin density from the neighboring carbon atom.[32] The angle between the p orbital on the carbon atom and the plane of the N–H bond will in this case be slightly greater than 2θ. In addition the value of Q^{H}_{NH} is expected to diminish with bending as in the case of Q^{H}_{CH} in the methyl group. Of course this calculation of bending angle should not be taken too seriously, since it relies on an acceptance of the theoretical parameters given in ref 12. If the parameters in ref 30 are accepted, the need to patch up the difference between experiment and theory does not seem so pressing. Also we wish to stress that the estimations of spin density, on which our treatment of splitting constants is partially based, are not foolproof. Lest the uncertainties in the theoretical Q matrix and the p matrix might seem to preclude any meaningful analysis of the splitting constants, we hasten to add that we feel the general form of our discussion is correct and that only minor changes in the matrices will be made necessary by further work.

Finally, the temperature dependence of the amine-group proton splittings remains unexplained. One difficulty in rationalizing this behavior is the fact that the nitrogen splitting is temperature independent. The decrease in a_H with rising temperature might indicate

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an increasing contribution of positive spin, possibly due to torsional displacement about the C–N bond.

Addendum

After this work was completed, we received a preprint from Professor H. Fischer describing a similar study by himself and Dr. H. Paul of radicals derived from amino acids and amides. There is excellent agreement between our experimental results, and they offer the same interpretation of the spectra as we do.[33]

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Electronic Spectra and Structures of Schiff's Bases. I. Benzanils[1]

M. Ashraf El-Bayoumi,*[2] M. El-Aasser,[3] and F. Abdel-Halim

Contribution from the Faculty of Science, Alexandria University, Alexandria, Egypt. *Received September 17, 1969*

Abstract: Electronic absorption spectra of various benzanils have been measured at room temperature in different media. Spectral changes due to substitution, change of solvent, and protonation support a noncoplanar structure for benzanils in which the nitrogen lone pair is conjugated to the phenyl group of the aniline part of the molecule. The spectra have been adequately interpreted in terms of two weakly interacting moieties of the molecule, namely the benzal and aniline parts. States have been identified as either locally excited or predominantly charge-transfer states. This classification has been useful in the assignment of the various electronic transitions.

A clear understanding of the electronic structure and spectral properties of Schiff's bases[4] is required for the explanation of their photochemical properties, phototropism, and photoisomerism. In particular, one needs to determine the nature of their lower electronic excited states, *e.g.*, the extent of charge-transfer character and the role of the solvent, protonation, and substitution in altering the energies of these states.

Schiff's bases contain the azomethine group $-CH{=}\ddot{N}-$, the same group that occurs in rhodopsin, the visual pigment extracted from rod cells. Rhodopsin consists of a retinal molecule (vitamin A aldehyde) and a large protein, opsin, linked together *via* the azomethine group. Moreover, Schiff's bases bear structural resemblance to stilbenes, azobenzenes, and several heterocyclic molecules; thus, a correlation of the electronic spectra of these compounds is useful in the assignment of their electronic transitions.

One aim of our study is to identify absorption bands as being due to intramolecular charge-transfer (CT) transitions involving the azomethine group or due to locally excited (LE) transitions. We also seek a confirmation of earlier evidence,[5-9] both experimental and theoretical, that benzylideneaniline is not coplanar and that π conjugation is interrupted at the nitrogen atom. If the molecule is planar, its π system would extend over both phenyl rings and the azomethine group, and the spectrum is expected to be similar to that of stilbene. However, if the molecule is noncoplanar, the spectrum should be more or less a superposition of the spectra of the two weakly interacting moieties of the molecule, namely the benzal and aniline parts.

The methods which we have used in our studies include substitution effects (both in the phenyl ring of the aniline part, Ph_N, and in the phenyl ring of the benzal part, Ph_C), solvent effects, and protonation effects on the spectra.

Experimental Section

Solvents were extensively purified to remove impurities that may interact with the solute molecules and to be spectrally transparent in the region down to 2000 Å.

Benzylideneaniline, benzylidene-*p*-hydroxyaniline, benzylidene-*p*-anisidene, *p*-hydroxybenzylideneaniline, *p*-hydroxybenzylidene-*p*-toluidine, *p*-hydroxybenzylidene-*p*-anisidene, *p*-methoxybenzylideneaniline, *p*-methoxybenzylidene-*p*-toluidine, *p*-methoxybenzylidene-*p*-anisidine, and *p*-*N*,*N*-dimethylaminobenzylideneaniline were prepared by warming (or refluxing, if required) equimolar amounts of the appropriate amine and aldehyde in alcohol. The products were recrystallized several times from alcohol.

Ultraviolet absorption spectra were measured at room temperature with a Zeiss PMQ II manual spectrophotometer using matched 1.00-cm fused-silica cells. All the spectra are plotted in terms of molar extinction coefficient ϵ *vs.* wavelength in Å.

Substituent Effects

Benzylideneaniline may be considered to have an electronic structure similar to that of stilbene, since the azomethine and vinyl groups are isoelectronic. One may therefore predict, at first consideration, similar absorption spectra for both molecules. Such expectations are not realized, however. Stilbene has an intense absorption band at 2950 Å (ϵ 24,000) and a less intense band at 2290 Å (ϵ 14,200), while the spec-

(1) Part of this work was carried out at the Biophysics Department, Michigan State University, under Contract No. AT(11-1)-2039, Division of Biology and Medicine, U. S. Atomic Energy Commission.

(2) Biophysics Department, Michigan State University, East Lansing, Mich. 48823.

(3) The major portion of this work is from the M.S. thesis of M. El-Aasser, Alexandria University, Sept 1966.

(4) Compounds containing the azomethine group $-CH{=}\ddot{N}-$ are given several names, *i.e.*, Schiff's bases, anils, azomethines, and benzylideneamines.

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