Electron Paramagnetic Resonance of Some γ-Irradiated Amino Acid Derivatives

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γ-Irradiated powders of *N*-acetyl-L-arginine, N_α-carbamyl-L-arginine, *N*-glycyl-L-leucine and glycyl-L-alanine were investigated at room temperature by electron paramagnetic resonance. The observed species in *N*-acetyl-L-arginine and N_α-carbamyl-L-arginine were attributed to the $CH_2\dot{C}HNHCNHNH_2$ radical, and those in *N*-glycyl-L-leucine and glycyl-L-alanine powders to $(CH_3)_2\dot{C}CH_2$ and $CH_3\dot{C}HCOOH$ radicals.

Key words: EPR; γ-Irradiation; Free Radicals; Amino Acid Derivatives.

1. Introduction

N-Acetyl-L-arginine (NALA), N_α-carbamyl-L-arginine (NCLA), *N*-glycyl-L-leucine (NGLL) and glycyl-L-alanine (GLA) are biologically important amino acid derivatives. Electron paramagnetic resonance (EPR) has been widely used for the identification of irradiation damage centers in many substance, including amino acids and their derivatives [1 – 11]. γ-Irradiated α-alanine, L-α-alanine, α-glycine and their derivatives were investigated at 4 K and 300 K [8 – 11]. The *g* values and hyperfine coupling constants of the unpaired electron with the environmental methyl, methylene, nitrogen and OH proton were determined. To our knowledge the title compounds have not been studied so far, and we have undertaken a study of the EPR spectra of these γ-irradiated compounds.

2. Experimental

The chemicals used in this study were obtained from commercial sources. The list of the amino acid derivatives studied with their chemical formulas and commercial names are presented in Table 1. First experiments were carried out with samples as received in quartz sample tubes. The powders were irradiated at room temperature with a ^{60}Co γ-ray source of 3 kGy h^{-1} for 5 h. The spectra were recorded with a Bruker EMX 081 model EPR spectrometer, using 5 mW microwave power. The *g* factors were calibrated by comparison with a DPPH sample ($g = 2.0036$). The γ-irradiated samples were stored at room temperature before recording the EPR spectra, and after the measurements the spectra were checked for two months to follow the stability of the formed species. The simulation of the spectra, using the EPR simulation program of McKelvey [12], are shown in Figs. 1b, 2b, 3b and 4b.

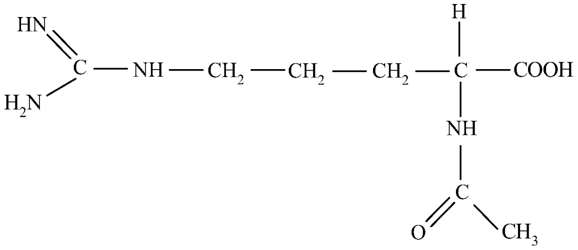
3. Results and Discussion

No EPR signal could be observed from unirradiated NALA, NCLA, NGLL and GLA. The observed species are usually produced by γ-irradiation and measured at room temperature. The EPR spectra of the radicals remained unchanged at room temperature for more than two months after irradiation. Some spectra are shown in Figs. 1 – 4, and the radicals attributed to these spectra are listed in Table 2.

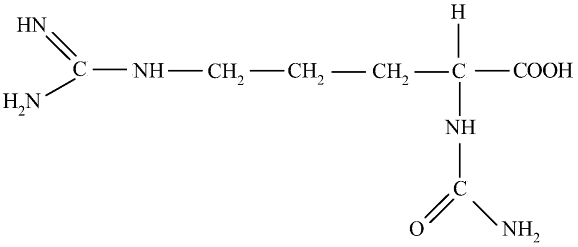
The EPR spectrum of the γ-irradiated NALA powder at room temperature is shown in Figure 1a. The spectrum consists of four lines with the intensity distribution 1 : 3 : 3 : 1, and the radical for this spectrum is thought to be the result of abstraction of one hy-

Table 1. List of the amino acid derivatives studied with their commercial names, chemical formulas, molecular weights (g/mol) and chemical structures.

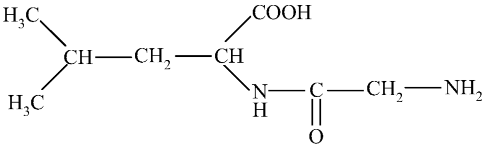
1. *N*-Acetyl-L-arginine, $C_8H_{16}N_4O_3$, 216.2



2. N_α-Carbamyl-L-arginine, $C_7H_{15}N_5O_3$, 217.2



3. *N*-Glycyl-L-leucine, $C_8H_{16}N_2O_3$, 188.3



4. Glycyl-L-alanine, $C_5H_{10}N_2O_3$, 146.15

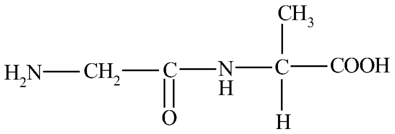
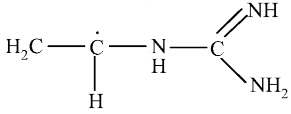
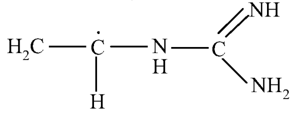


Table 2. Precursor number, EPR parameters and proposed structure of irradiation-produced radical species.

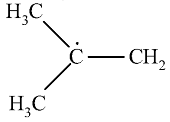
1. $g = 2.0027$,
$a_\alpha = 1.80$ mT, $a_\beta = 2.48$ mT, $a_N = 0.20$ mT, $\Delta H = 0.35$ mT



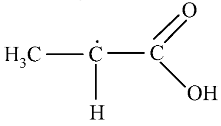
2. $g = 2.0028$,
$a_\alpha = 2.05$ mT, $a_\beta = 0.93$ mT, $a_N = 0.37$ mT, $\Delta H = 0.30$ mT



3. $g = 2.0028$,
$a_\beta^{(1)} = 1.36$ mT, $a_\beta^{(2)} = 0.82$ mT, $\Delta H = 0.34$ mT



4. $g = 2.0028$,
$a_\alpha = 1.89$ mT, $a_\beta = 1.89$ mT, $a_{OH} = 0.45$ mT, $\Delta H = 0.30$ mT



drogen atom from the α-carbon atom (Table 2). The unpaired electron couples with two equivalent methylene protons and one proton directly attached to the α-carbon atom and nitrogen nucleus, which are all approximately magnetically equivalent.

The spectrum for a larger number of unequivalent protons can be found by graphical construction or by binomial expansion, which is a mathematical record of an EPR spectrum. Simple addition of the line intensities of simple spectra, the lines being displaced relative to each other by the value of the coupling constant, yields the intensity ratio for more complicated spectra (see binomial expansion). This spectrum, corresponding to the binomial expansion $1:2:1+1:2:1 = 1:3:3:1$, is shown in Figure 1a. A simulation of the NALA spectrum is shown in Fig. 1b, using the hyperfine coupling constants $a_\alpha = 1.80$ mT, $a_\beta = 2.48$ mT and $a_N = 0.20$ mT. a_α is smaller than a_β in this compound. The difference in the coupling constants of the compounds which have similar structure may be due to the difference in their conformation. Some examples are given in Table 3. The measured value of the g factor is $g = 2.0027 \pm 0.0005$. The hyperfine constants and the g value of the radical discussed here seem to agree well with the literature values of their analogous and various other amine radicals [13–15]. The linewidth of the spectrum is somewhat larger than the hyperfine coupling constant of the nitrogen nuclei ($a_N = 0.2$ mT), and therefore the hyperfine splitting of the nitrogen nuclei was not observed in the spectrum. There is good agreement between the experimental and simulated EPR spectra. The radical is similar to the amine radical.

The γ-irradiated powder of NCLA at room temperature gives the spectrum shown in Figure 2a. This spectrum can be attributed to the radical shown in Table 2. The spectrum can again be interpreted as a $1:2:1$ triplet of a $1:1$ doublet. With the hyperfine constants $a_\alpha = 2.05$ mT, $a_\beta = 0.93$ mT, and $a_N = 0.37$ mT the g factor is $g = 2.0028 \pm 0.0005$. The hyperfine constants and the g value are similar to those for amine radicals. When the spectrum is examined thoroughly, it can be seen that it consists of a doublet with a spac-

Table 3. EPR parameters of some primary, secondary and tertiary amines.

Amine	Radical	Hyperfine coupling constant (mT)				
		a_{α}^{H}	a_{β}^{H}	a^{N}	$a_{NH_2}^{H}$	*g* values
Ethylamine	$CH_3\dot{C}HNH_2$	1.53	2.02	0.49	0.49	2.0027
n-Propylamine	$CH_3CH_2\dot{C}HNH_2$	1.455	1.643	0.475	0.475	2.0026
Isobutylamine	$(CH_3)_2CH\dot{C}HNH_2$	1.410	1.875	0.465	0.465	2.0029
Diethylamine	$CH_3CH_2NH\dot{C}HCH_3$	1.46	2.00	0.48	0.09	2.0027
Triethylamine	$(C_2H_5)_2N\dot{C}HCH_3$	1.365	1.958	0.51	–	2.0031
Trimethylamine	$(CH_3)_2N\dot{C}H_2$	1.335	–	0.37	–	2.0028
Ethyl-*n*-propylamine	$C_2H_5CH_2NH\dot{C}HCH_3$	1.47	2.01	0.52	~ 0	2.0027

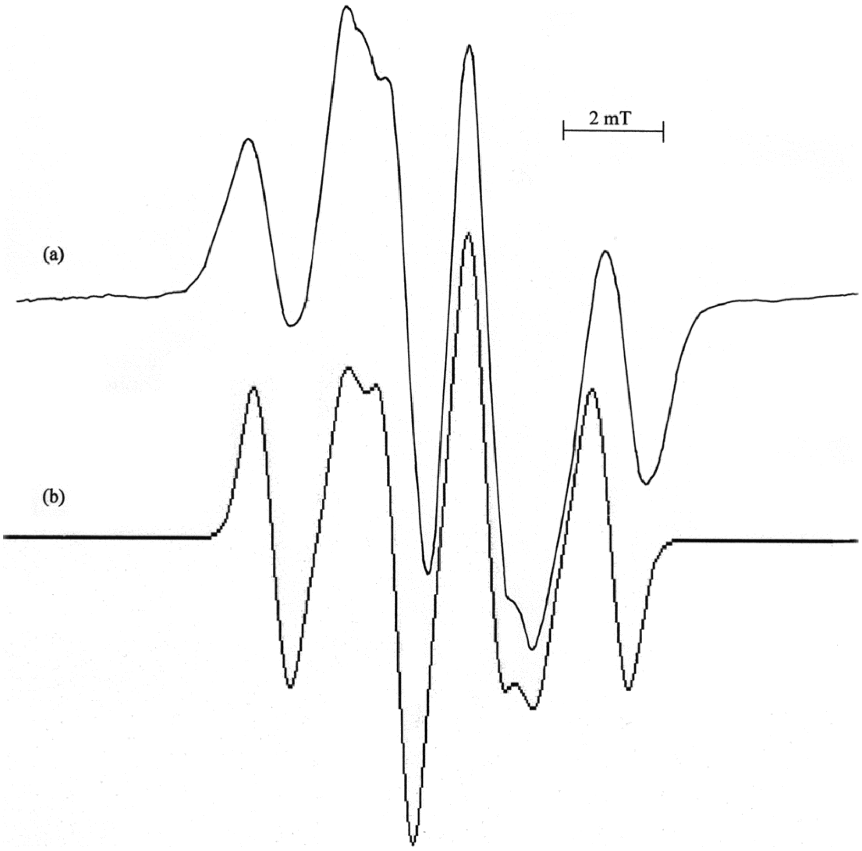


Fig. 1. (a) EPR spectrum of γ-irradiated *N*-acetyl-L-arginine powder; (b) simulation of the spectrum.

ing of 2.05 mT. Then each line of the doublet is further subdivided into 3 lines of spacing 0.93 mT, with an intensity distribution of 1 : 2 : 1, as shown Figure 2a. These inferences indicate the hyperfine coupling of the unpaired electron with one α-proton and two methylene protons. The N nucleus is not resolvable due to the smallness of the nitrogen coupling constant. A spectrum simulated with these values agrees well with the experiment (Fig. 2b). In conclusion it can be stated that γ-irradiation produces free radicals in NCLA by loss of one hydrogen atom from the CH_2CH_2 group.

The EPR spectrum of γ-irradiated NGLL is shown in Figure 3a. The spectrum may consist of 5 lines with the intensity distribution 1 : 4 : 6 : 4 : 1. The unpaired electron interacts with two equivalent methylene protons and two protons of two methyl groups, which are all magnetically equivalent, and shows the intensity distribution of five lines. Therefore the paramagnetic center can be attributed to the radical shown in Table 2; it may be a result of removal of a proton from the carbon atom attached to the methylene group. The *g* value, hyperfine constants and line width obtained by the simulation are shown in Table 2. The reported $g = 2.0026$ for pure L-leucine methyl ester [16] at room temperature agrees well with our $g = 2.0028 \pm 0.0005$. The hyperfine interactions with the protons are $a_{\beta}^{(1)} = 1.36$ mT

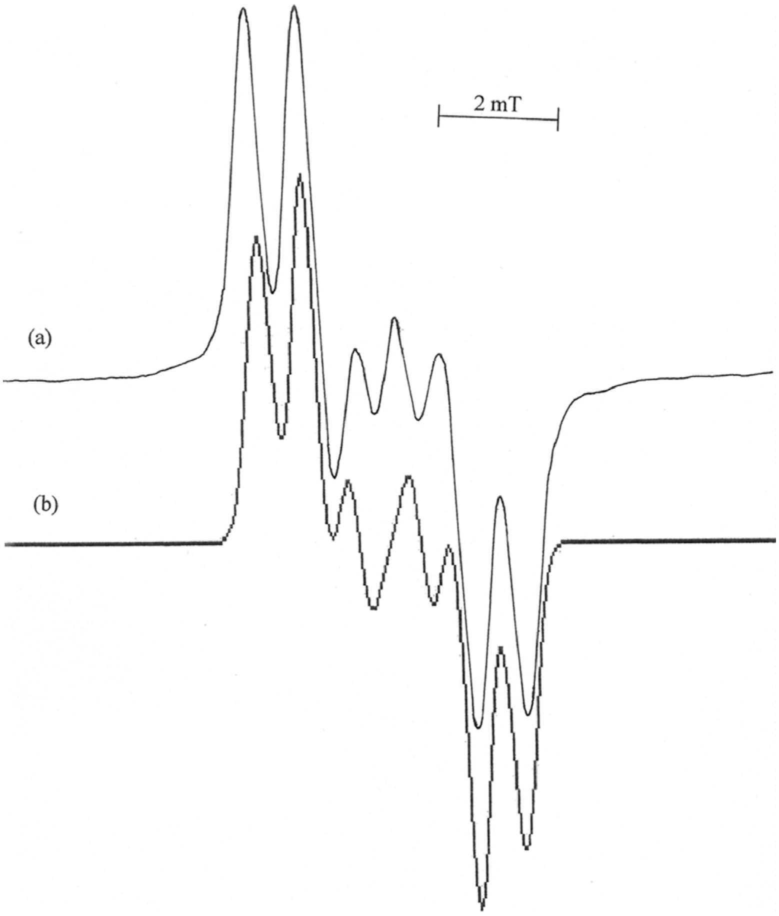


Fig. 2. (a) EPR spectrum of γ-irradiated N_α-carbamyl-L-arginine powder; (b) simulation of the spectrum.

and $a_\beta^{(2)} = 0.82$ mT. These results are similar to those reported by Kartal et al. [16]. A spectrum simulated with these values agrees well with the experiment and is shown in Figure 3b. A radical similar to this has been observed in the γ-irradiated L-leucine methyl ester and $Al_6O_4(OH)_{10}(leucine) \cdot 5H_2O$. But the hyperfine coupling constants of the methylene protons reported here are smaller than the values reported in [17]. These differences in the methylene proton coupling constants can be understood from the hyperfine coupling constant relation [9] of the β-protons. This can be ascribed to the fact that β-coupling is angle-dependent as expressed by

$$a_\beta = B_0 + B_1 \cos^2 \theta. \qquad (1)$$

In the case of rapid rotation about the C_α-C_β bond the average value becomes

$$a_\beta = B_0 + \frac{1}{2} B_1, \qquad (2)$$

where B_0 is the spin polarisation contribution ($0 < B_0 < 0.35$ mT) [9] and B_1 the hyperconjugative contribution ($B_1 = 3.21$ mT) [18]. We obtain $a_\beta = 1.6$ mT; this is in good agreement with our experimental result, 1.36 mT. Hence we conclude that the β-protons in the $(CH_3)_2\dot{C}CH_2$ radical rotate about the C_α-C_β bond.

The EPR spectrum at room temperature of γ-irradiated GLA powder was obtained as shown in Figure 4a. The spectrum consists of five lines of the intensity ratio 1 : 4 : 6 : 4 : 1 with 1.89 mT spacing. This pattern is obviously due to the hyperfine interaction of the unpaired electron with three methyl protons and one α-proton, which are all magnetically equivalent. The spectrum in Fig. 4a shows five lines partly due to the broadness of the lines, and partly due to the smallness of the OH coupling. A simulation of the spectrum is shown in Figure 4b. The experimental and simulated EPR spectra were found to agree well with each other. The measured value of the g factor and hyper-

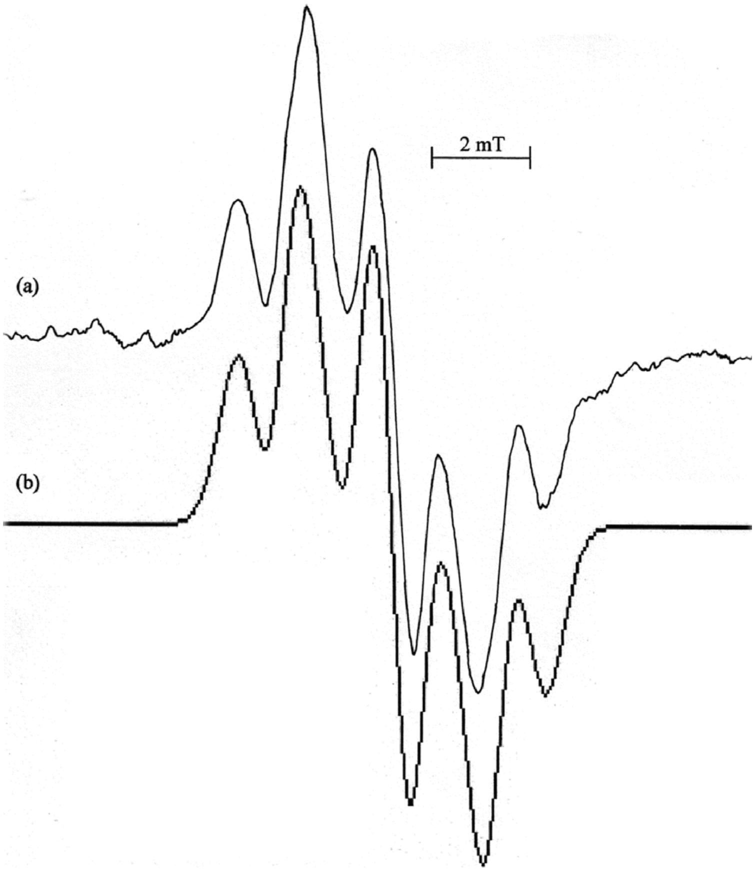


Fig. 3. (a) EPR spectrum of γ-irradiated *N*-glycyl-L-leucine powder; (b) simulation of the spectrum.

fine constants are $g = 2.0028 \pm 0.0005$, $a_\alpha = 1.89$ mT, $a_\beta = 1.89$ mT, $a_{\mathrm{OH}} = 0.45$ mT, respectively. These values agree well with some other literature data [8–11, 14]. Single crystals of the γ-irradiated amino acid L-α-alanine have been studied with X-ray at room temperature. The radiation damage center of this sample was attributed to the $CH_3\dot{C}HCOOH$ radical [19–22]. This radical is similar to radical obtained in glycyl-L-alanine.

γ-Irradiation produces some stable aminoalkyl- and amine-type free radicals in some amino acids and derivatives. The hyperfine constants of the radicals discussed here seem to agree well with the literature values for their analogues and various other alkyl and amine radicals [9–11, 15, 16]. The spectra of rapidly reorienting free radicals in adamantane are isotropic. Linewidths of 0.15 and 0.2 mT were observed for the aminoalkyl radicals in adamantane [15]. In the present study the linewidths vary between 0.30 and 0.35 mT, and some other radicals seem to do the same. The values of the hyperfine coupling constants of the free electron of the α- and β-protons and nitrogen nuclei in primary, secondary and tertiary amine radicals are of the same order as in this study. Although the line resolution is poor, the radicals could be identified, and EPR parameters could be determined.

Acknowledgements

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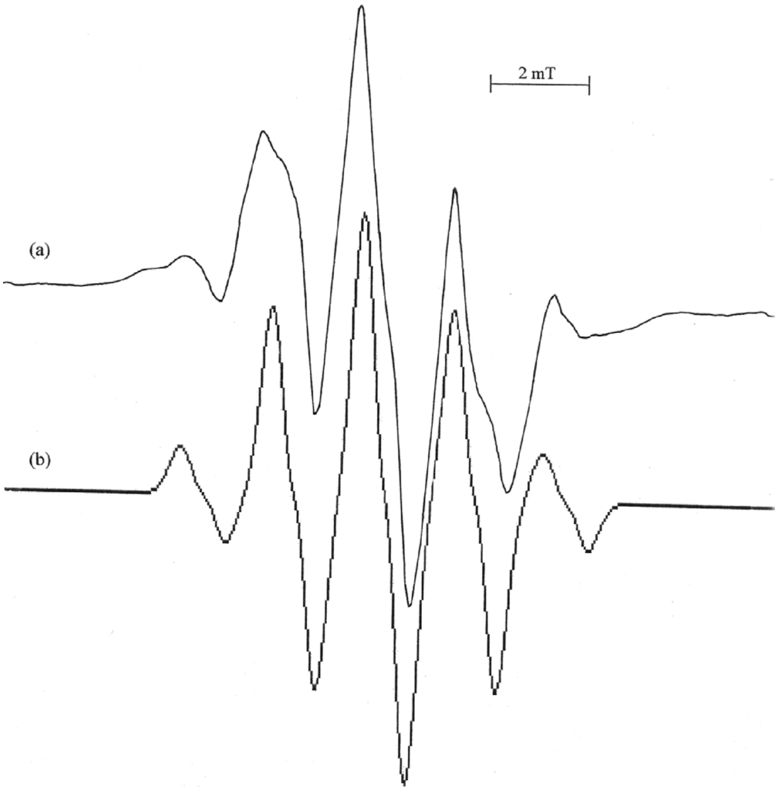

Fig. 4. (a) EPR spectrum of γ-irradiated glycyl-L-alanine powder; (b) simulation of the spectrum.

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