Indian Journal of Pure & Applied Physics
Vol. 48, September 2010, pp. 611-614

EPR study of free radicals in amino acid derivatives gamma-irradiated at 300 K

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Received 28 January 2010; revised 2 July 2010; accepted 9 August 2010

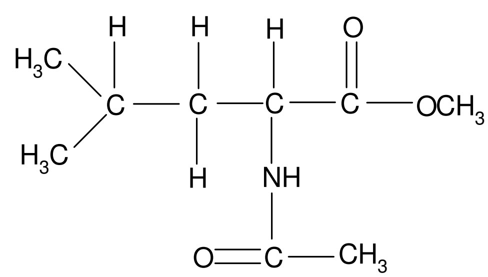
Electron paramagnetic resonance spectroscopy has been used to study the free radicals in N-acetyl-L-leucine methyl ester and N-acetyl-L-glutamic acid powders gamma-irradiated at room temperature (300 K). It has been found that gamma-irradiation produces the $(CH_3)_2\dot{C}CH_2CH(NHCOCH_3)COOCH_3$ radical in the first and the $HOOCCH_2CH_2\dot{C}(NHCOCH_3)COOH$ radical in the second compounds. The *g* values of the radicals and the hyperfine coupling constants of the free electron with the environmental methyl, methylene protons and ^{14}N nucleus have been determined. Both free radicals are found to be stable without almost intensity diminution for five months at room temperature. The results have been found to be in good agreement with the existing literature data.

Keywords: EPR, Amino acid derivatives, Gamma-irradiation, Free radicals

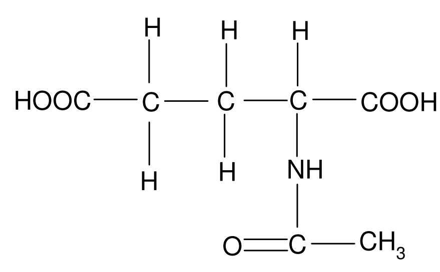
1 Introduction

Free radicals are important constituents of many reaction mechanisms[1] and they significantly contribute to normal functioning of the organisms as well as development of pathological process[2]. It is known that the free radicals are important mediators of a wide range of clinical diseases such as heart attack, rheumatism, cardiovascular diseases, stroke, and cancer[3]. There are two ways of creation of free radical components within the biological systems: (a) metabolic, also known as natural and (b) resulted due to external factors[2]. Gamma-irradiation is considered to be one of such external factors. Since in free radicals, the unpaired electron is involved, these species are paramagnetic and the most used method for detecting free radicals is electron paramagnetic resonance (EPR) spectroscopy[4]. Therefore, EPR spectroscopy has been widely used for the identification of irradiation damage centers in many substances including amino acid and their derivatives[5-13]. The gamma-irradiated single crystals of N-acetyl-L-leucine were investigated at room temperature by EPR and the radiation damage centers were attributed to the $(CH_3)_2\dot{C}CH_2CH(NHCOCH_3)$-COOH and $(CH_3)_2CHCH_2\dot{C}(NHCOCH_3)COOH$ radicals[12]. Furthermore Osmanoglu *et al.*[15] showed that $HOOCCH_2CH_2\dot{C}(NHCONH_2)COOH$ radical were observed in gamma-irradiated N-carbamoyl-L-glutamic acid at room temperature.

Leucine and glutamic acid are biologically important amino acids which play an essential role in the metabolic processes. In this paper, stabilities of the radical species formed in N-acetyl-L-leucine methyl ester (NALME) (**1**) and N-acetyl-L-glutamic acid (NALGA) (**2**) powders have been identified and checked by gamma-irradiation at room temperature.



(**1**)



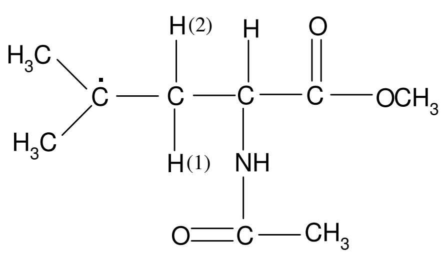
(**2**)

2 Experimental Details

The samples used in this study were obtained from commercial sources. Powder samples of the compounds were exposed to gamma-irradiation from ^{60}Co gamma-ray source at dose rate of 2 kGy/h for a total of 12 h at room temperature. After irradiation, samples were kept in plastic bags at room temperature in the dark. The spectra of samples were recorded by putting the sample in quartz sample tube. The EPR measurements were carried out in a Varian model X-band E-109C EPR spectrometer at room temperature. The modulation amplitude was below 5.10^{-2} mT and the microwave power was 2 mW. The spectrometer frequency was corrected using the DPPH (dipenhylpicrylhydrazyl, g = 2.0036) sample. The spectrum simulations were made using McKelvey's programs[16].

3 Results and Discussion

While unirradiated NALME samples exhibit no resonance signal, samples irradiated at room temperature are observed to present EPR spectra with many resonance peaks as shown in Fig. 1(a). When the spectrum is examined thoroughly, it can be seen that it consists of a doublet with a spacing of 3.23 mT. Then each line of doublet is further subdivided into 7 lines of spacing 2.30 mT with an intensity distribution of 1:6:15:20:15:6:1. These seven lines are also doublets, with a splitting constant of 0.86 mT. These inferences indicate the hyperfine couplings of the unpaired electron with six equivalent protons of two methyl groups and the protons of a methylene group. The hyperfine splitting constants of the methylene protons are measured as $a_\beta^{(1)}$ = 3.23 mT and $a_\beta^{(2)}$ = 0.86 mT. Therefore, we may assume that this EPR spectrum belongs to the radical (R_1) given below:



(R_1)

The g value of the R1 is measured as g = 2.0035 ± 0.0005. A simulation of the spectrum is shown in Fig. 1(b), using the hyperfine coupling constants $a_{(CH_3)_2}$ = 2.30 mT, $a_\beta^{(1)}$ = 3.23 mT and $a_\beta^{(2)}$ = 0.86 mT. The experimental and simulated EPR spectra are found to agree well with each other. Almost identical spectrum was obtained after the powder of L-leucine methyl ester hydrochloride subjected to gamma-irradiation[17]. The radical determined is similar to our proposed radical and reported values of $a_{(CH_3)_2}$ = 2.30 mT, $a_\beta^{(1)}$ = 3.80 mT and $a_\beta^{(2)}$ = 0.90 mT are in good agreement with our results. In another study, a radical similar to this has been observed in gamma-irradiated $Al_6O_4(OH)_{10}(leucine)_2{\cdot}5H_2O$ system[16], but the hyperfine coupling constant of one of methylene protons ($a_\beta^{(1)}$ = 2.30 mT and $a_\beta^{(2)}$ = 0.70 mT) is smaller than the value reported here. Almanov *et al.*[19] have reported the EPR investigation of gamma-irradiated N-formyl-L-leucine single crystal at room temperature and they have determined $(CH_3)_2\dot{C}CH_2CH(NHCOH)\text{-}CO_2$ radical. They also reported values of the hyperfine constants as $a_{(CH3)2}$ = 2.40 mT and $a_\beta^{(1)}$ = 2.40 mT, and in their study, it was observed that the unpaired electron did not interact with the second $\beta^{(2)}$-proton of methylene group. The g value and the hyperfine constants of methyl protons of the radical discussed here agree well with earlier data[20,21].

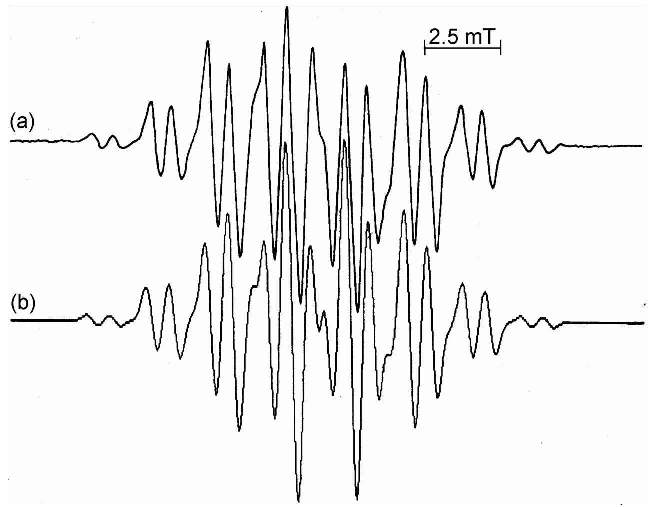


Fig. 1 — (a) The EPR spectrum of gamma irradiated NALME powder at room temperature, (b) simulation form of the spectrum using $a_{(CH3)2}$ = 2.30 mT, $a_\beta^{(1)}$ = 3.23 mT, $a_\beta^{(2)}$ = 0.86 mT and linewidth 0.27 mT

The characteristic EPR spectrum of the gamma-irradiated NALGA is shown in Fig. 2(a). When the spectrum is examined thoroughly, it consists of a doublet with a spacing of 2.83 mT. Since the linewidth of the spectrum is large, the hyperfine splitting of the nitrogen nuclei is not observed in the spectrum. But, it is obtained using the simulation program in a_N=0.43 mT. Similar values of the hyperfine coupling constants and the spectrum have been found in the gamma-irradiated N-carbamoyl-L-glutamic acid single crystal as $a_\beta^{(1)}$ = 2.76 mT and a_N=0.43 mT (Ref. 15). These indicate the hyperfine

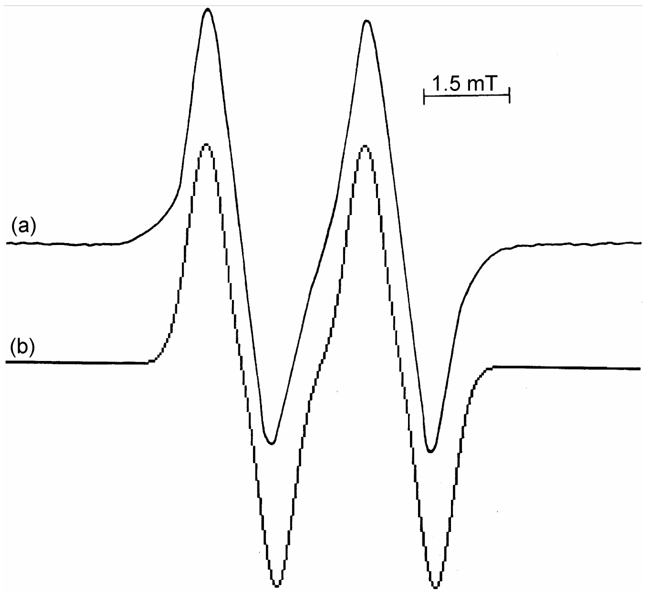
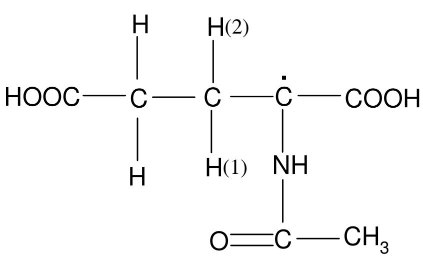


Fig. 2 — (a) The EPR spectrum of gamma irradiated NALGA powder at room temperature, (b) simulation form of the spectrum using $a_\beta^{(1)} = 2.83$ mT, $a_N = 0.43$ mT and linewidth 0.40 mT

coupling of the unpaired electron with one of the methylene protons ($a_\beta^{(1)} = 2.83$ mT) and a ^{14}N nucleus (a_N=0.43 mT). Hence, the paramagnetic centre can be attributed to the radical (**R2**).



(**R2**)

The measured g value of the R2 is g = 2.0030 ± 0.0005. A simulated spectrum, with the above given values, agrees well with the experiment Fig. 2(b). These values of the hyperfine coupling constants are in agreement with the earlier values on some amine radical[22,23]. Single crystal of the gamma irradiated Nα-acetyl L-glutamic acid has been investigated at room temperature and the radiation damage centre of this sample has attributed to the HOOCCH$_2$CH$_2$Ċ-(NHCOCH$_3$)COOH radical[24]. The radical is similar to our proposed radical, but the hyperfine coupling constant of $\beta^{(1)}$-proton (3.65 mT) is higher than the value reported here.

In the R2, only one proton of the methylene group shows a resolvable coupling, because the hyperfine coupling constant of second proton of the methylene group is under 0.4 mT (ref. 24). The magnitude of the β-proton coupling constant depends on the dihedral angle θ between the C-H bond and it is given[25] as:

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

In this equation, the B_0 is a constant and includes the contribution of spin density which arises from conformation independent mechanisms, in particular spin polarizations, and B_1 includes the hyperconjugative contributions. In the case of rapid free rotation about C_α–C_β the average value of a_β becomes:

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

with B_0 = 0-0.35 mT and B_1 = 5.00 mT, which was earlier measured[25]. If these values are replaced in (2), a_β = 2.50-2.85 mT is obtained. The value $a_\beta^{(1)}$ = 2.83 mT obtained in this study is in this range. Therefore we conclude that the β-proton in the R2 radical rotates about C_α–C_β. We can state that this rotation of the β-proton exists at room temperature.

4 Conclusions

It can be concluded that gamma-irradiation produces very stable alkyl and amine type free radicals in amino acid derivatives. Also, it can be stated that the R1 radical is obtained by the removal of hydrogen atom from the tertiary carbon atom of the NALME and R2 radical is obtained by the removal of hydrogen atom from the carbon atom bond to the NHCOCH$_3$ and the COOH group in the NALGA. Although, the line resolution is poor, the radicals could be identified and the EPR parameters could be determined. The measurements of magnetic properties of these radicals can be helpful for studies of similar radicals found in biological systems.

Acknowledgement

This work was supported by Grant No. EFBAP2009-0005 of the Research Fund of Adiyaman University (ADÜYAP).

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