Bulgarian Chemical Communications, Volume 49, Number 1, (pp. 82 – 86) 2017

Free radicals properties of some gamma-irradiated organic compounds

R. Şimşek [1], M.G. Gündüz [1], C. Şafak [1], Ö. Kökpınar[2], M. Aydın[3*]

[1]*Faculty of Pharmacy, Hacettepe University, TR 06100-Ankara, Turkey*
[2]*Vocational School of Technical Sciences, Adıyaman University, Turkey*
[3]*Faculty of Education, Adıyaman University, TR 02040-Adıyaman, Turkey*

Received May 15, 2015; Revised February 3, 2016

Powders of some organic compounds (compounds 1-4) were irradiated with ^{60}Co gamma-rays and the spectroscopic properties of the free radicals formed were investigated at ambient temperature using electron paramagnetic resonance (EPR) technique. The analysis of the EPR spectra of irradiated powder samples demonstrated that the paramagnetic species produced by the radiation damage was $N\dot{C}H_2$. The *g* values of the radicals and the hyperfine structure constants of the free electron with nearby protons and ^{14}N nucleus were determined. The EPR spectra show that they are of radicals remained unchanged for more than two months. The results were found to be in good agreement with earlier studies on amine radicals.

Keywords: EPR, Free radicals, Gamma-irradiation, 1,4-dihydropyridines.

INTRODUCTION

It is known that ionizing radiations produce free radicals that have unpaired electron [1]. Free radicals provoke both beneficial and harmful effects in living organisms [2]. By initiating and propagating free radical chain reactions, they can damage the cells and may be involved in the progression of a number of diseases including diabetes, hyperlipidemia, neurodegeneration, heart disease, cancer, and so on [3-5]. Qualitative and quantitative aspects of free radicals in oxidative stress can be evaluated by using different techniques [6]. The EPR spectroscopy is the most effective technique for detection of free radicals [7, 8]. Thus, the EPR method has been applied to the identification of irradiation damage centres in many substances, including amino acids, drugs and other organic compounds [9-18]. The spectroscopic splitting factor *g* and the hyperfine coupling constants (*a*) of unpaired electrons give valuable information about the structure of the radical [19]. The X-irradiated hippuric acids have been investigated at 295 K by the EPR technique and the observed paramagnetic species have been attributed to the $PhCONH\text{-}\dot{C}H_2$ radical [20]. Furthermore, the EPR of gamma-irradiated powders of L-arginine monohydrochloride were studied by Aydin [21] at ambient temperature, and the radiation damage center was attributed to the $NH_2(NH)HN\dot{C}HCH_2CH_2CH(NH_2)COOH$ radical. Another technique to identify the molecular structure of substances is the infrared spectroscopy.

1,4-Dihydropyridines are an important class of L-type calcium channel blockers that mainly exert their pharmacological activity by modulating Ca^{2+} influx and are used to treat cardiovascular conditions such as hypertension and angina [22, 23]. We have previously reported the development of a series of condensed 1,4-dihydropyridine derivatives. Although the effects of these compounds on calcium and potassium channels have been elucidated [24, 25], the defects induced by gamma-irradiation have not been investigated. The purpose of this study is to investigate the defects induced by gamma-irradiation in powders of 1,4-dihydropyridine derivatives (compounds 1-4) and to determine their properties at ambient temperature.

EXPERIMENTAL

Materials

The study was performed on the following dihydropyridine derivatives differing in number, position and type of the substituents:

Compound 1: methyl 2-methyl-4-(2,3-dichlorophenyl)-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate; molecular weight (MW, g/mole): 442.34;

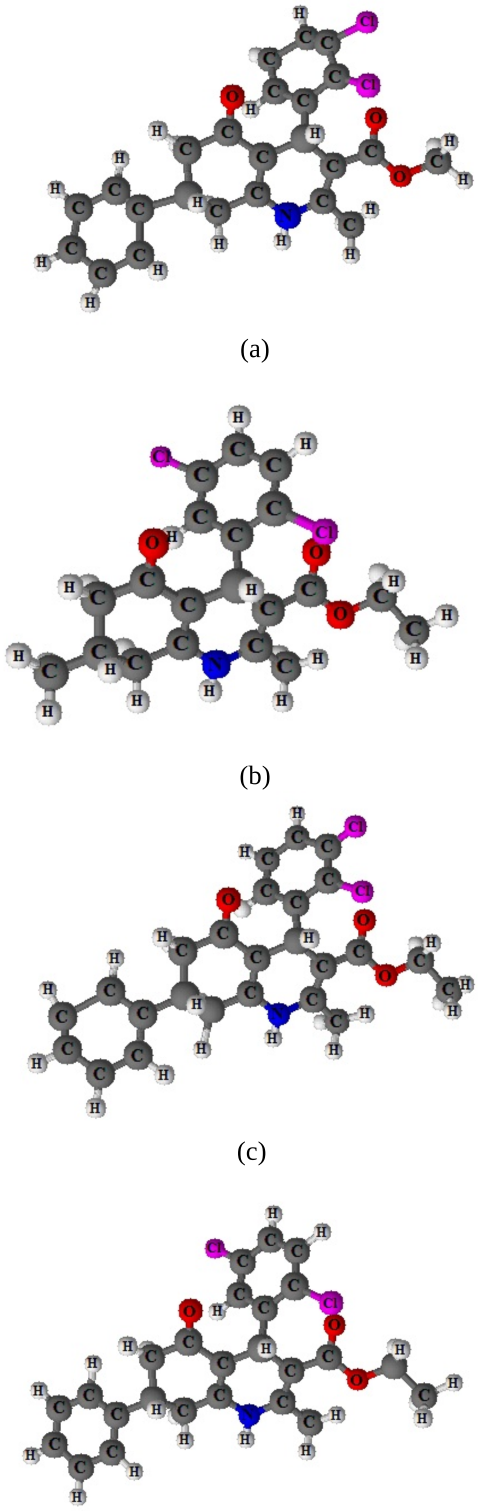
Compound 2: ethyl 2,7-dimethyl-4-(2,5-dichlorophenyl)-5-oxo-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate; MW, g/mole: 394.30;

Compound 3: ethyl 2-methyl-4-(2,3-dichlorophenyl)-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate; MW, g/mole: 456.37;

Compound 4: ethyl 2-methyl-4-(2,5-dichlorophenyl)-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate; MW, g/mole: 456.37.

---

* To whom all correspondence should be sent:
E-mail: maydin@adiyaman.edu.tr



(a)

(b)

(c)

(d)

Fig. 1. Molecular structures of: (a) compound **1**, (b) compound **2**, (c) compound **3**, (d) compound **4.**

The synthetic procedure to obtain the compounds 1-4 has been described previously [24]. Briefly: 1 mmol of the appropriate 1,3-cyclohexanedione (5-methyl-1,3-cyclohexanedione /5-phenyl-1,3-cyclohexanedione), dichloro-substituted benzaldehyde (1 mmol) and methyl or ethyl aminocrotonate (1 mmol) were refluxed for 4 h in methanol. The solvent was then evaporated and the residue was crystallized from ethanol. The molecular structures of the compounds are shown in Figs. 1a-d which are simple demonstration pictures obtained from ACD/3D Viewer (Freeware) to explain the structural properties of the compounds used in this study.

Irradiation

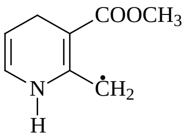
The powder samples were irradiated for 40 h using ^{60}Co gamma ray source with dose speed of 0.89 kGy/h. The irradiated samples were kept in plastic bags at ambient temperature in the dark.

EPR spectroscopy

The EPR measurements were performed with a Varian model X-band E-109C spectrometer at ambient temperature. The modulation amplitude was below 0.40 mT and the microwave power was 2 mW. The spectroscopic g factors of the radicals were determined by comparison with a dipenhylpicrylhydrazyl (DPPH) sample (g = 2.0036) as reference [26]. Before irradiation no EPR signals were observed for all studied samples. The EPR spectra of the gamma-induced free radicals were unchanged and undiminished at ambient temperature for more than two months after irradiation.

RESULTS AND DISCUSSION

The EPR spectrum of the gamma irradiated compound 1 at ambient temperature is shown in Fig. 2a. The spectrum exhibits a triplet (1:2:1) due to the two equivalent α - protons adjacent to the carbon atom and each of the lines (1:2:1) splits into the triplet (1:1:1) because of the ^{14}N nucleus, resulting in an approximate intensity distribution of 1:1:1:2:2:2:1:1:1. Hence, the paramagnetic species can be attributed to the R1 radical which is a result of the removal of hydrogen atom from the methyl group.



(R1)

The EPR parameters (g factor and hyperfine splitting) of the R1 radical were extracted from the EPR spectrum analysis as a_{α} = 1.56 mT, a_N = 0.42 mT and g = 2.0034 ± 0.0005. The simulation of the EPR spectrum with the above given values is shown in Fig. 2b.

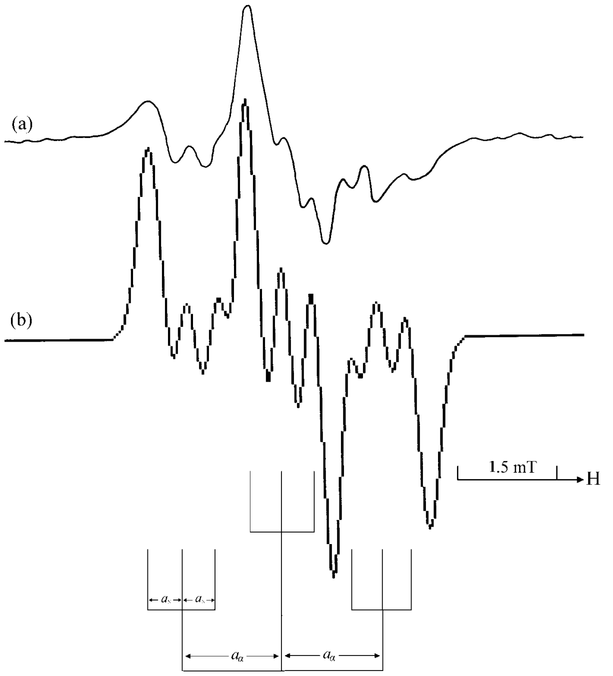


Fig. 2. (a) EPR spectrum of gamma-irradiated compound **1**, (b) simulation of the spectrum.

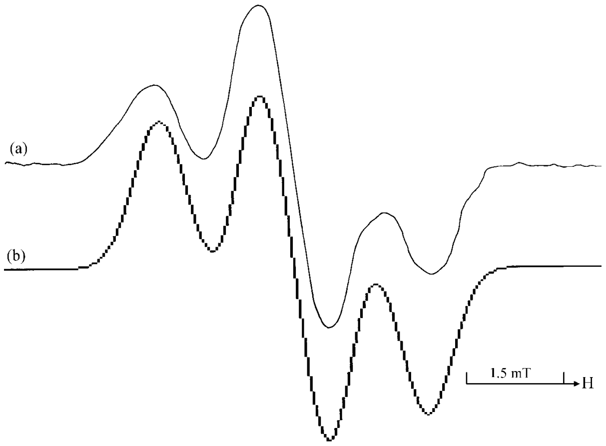
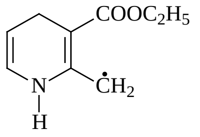


Fig. 3. (a) EPR spectrum of gamma-irradiated compound **2**, (b) simulation of the spectrum.

The radical R1 discussed here is similar to the radical which has been observed in the gamma-irradiated powders of ethyl 2-methyl-4-(2,6-dichlorophenyl)-5-oxo-7-phenyl-1,4,5,6,7,8-hexahydroquinoline-3-carboxylate at ambient temperature; the determined EPR parameters were $a_\alpha = 1.24$ mT, $a_N = 0.48$ mT and $g = 2.0029$ [27]. But the hyperfine coupling constants of the α- protons measured here are higher than the values reported in ref. [27]. Similar values of the hyperfine coupling constants of α- protons have been found in the gamma-irradiated N-glycyl-L-valine as 1.45 mT [28].

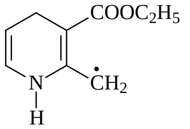
The characteristic EPR spectrum of the gamma-irradiated compound 2 at ambient temperature is shown in Fig. 3a. As can be seen, the spectrum consists of a triplet with a spacing of 1.34 mT. The 1:2:1 triplet structure of the spectrum is obviously brought about by the interaction of the unpaired electron with two magnetically equivalent α- protons in the methylene group. Therefore, we assume that this EPR spectrum belongs to the R2 radical.



(R2)

A simulation of the EPR spectrum is shown in Fig. 3b using the hyperfine coupling constants a_α = 1.34 mT, a_N = 0.41 mT and linewidth (ΔH) 0.54 mT. Since the linewidth of the spectrum is larger than the hyperfine splitting of the nitrogen nuclei, the hyperfine splitting is not observed in the spectrum and was determined using the simulation program. The g value of the radical was measured as $g = 2.0029 \pm 0.0005$. The EPR spectrum of the radical R2 is similar to the EPR spectrum observed in the irradiated powders of ammonium acetate at ambient temperature [4], but the hyperfine coupling constants of α-protons were higher than the values determined for the radical R2. However, similar to the R2 radical were the hyperfine coupling constants of α-protons measured in the gamma- irradiated azocalix [4] arene (a_α = 1.28 mT) [29].

Fig. 4a presents the EPR spectrum of the gamma-irradiated compound 3, recorded at ambient temperature. Since this spectrum has fairly large linewidth (ΔH= 0.60 mT), it is not resolvable at first view. The EPR parameters of the spectrum can be determined from the simulation of the spectrum [30, 31]. The hyperfine constants values obtained by the simulation are a_α = 1.24 mT, a_N = 0.46 mT and ΔH= 0.60 mT and the simulated spectrum (Fig. 4b) is in excellent agreement with the experimental spectrum. The measured g value at the center of the spectrum is 2.0032. According to these results, we can state that the R3 radical is obtained by the removal of hydrogen from the methyl group.



(R3)

The EPR spectrum of the gamma-irradiated compound 4 is similar to that of the irradiated compound 3. Thus, the observed radical of the gamma-irradiated compound 4 is similar to the radical R3. Therefore, the spectrum of compound 4 is not shown. The g values, hyperfine coupling constants and linewidth obtained by the simulation are a_α = 1.24 mT, a_N = 0.46 mT and g = 2.0027 ± 0.0005. These results agree well with the EPR parameters of the $(CH_3)_2N\dot{C}H_2$ radical [12]. The g values of the radicals in this study, which were produced by gamma rays, are in agreement with the literature data for their analogous and various other amine radicals [15, 21, 27, 32-36].

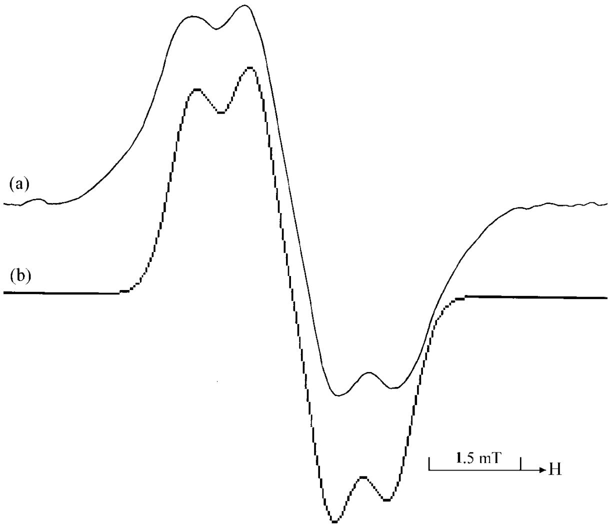


Fig. 4. (a) EPR spectrum of gamma-irradiated compound **3** and compound **4,** (b) simulation of the spectrum.

CONCLUSION

It can be concluded that gamma-irradiation produces very stable amine type free radicals of dihydropyridine derivatives. The EPR parameters and the spectroscopic properties of these radicals were determined. The values of the spectroscopic splitting factor g of these radicals were quite similar to the g-factor for a free electron. The determination of magnetic properties of the produced radicals can be helpful in the study of similar radicals found in biological systems.

REFERENCES

1. H. Tuner, M.O. Bel, M. Polat, *Radiat. Phys. Chem.*, **107**, 115 (2015).
2. S.I. Rana, R. Chawla, R. Kumar, S. Singh, A. Zheleva, Y. Dimitrova, V. Gadjeva, R. Arora, S. Sultana, R. K. Sharma, *J. Pharm. Bioallied Sci.*, **2**, 80 (2010).
3. U. Sayin, Ö. Dereli, E. Türkkan, H. Yüksel, M. Birey, *Radiat. Phys. Chem.*, **80**, 38 (2011).
4. M. H. Başkan, M. Aydın, D. Çanakçı, Ş. Osmanoğlu, *Radiat. Eff. Defect. Solids*, **169**, 226 (2014).
5. X. Guo, C. Chang, E. Y. Lam, *J. Magn. Reson.*, **204**, 26 (2010).
6. C. L. Hawkins, M. J. Davies, *Biochim. Biophys. Acta,* **1840**, 708 (2014).
7. B. Calıskan, H. Tokgoz, *Radiat. Eff. Defect. Solids***, 169**, 225 (2014).
8. B. Calıskan, *Acta Phys. Pol. A***, 125**, 135 (2014).
9. W. C. Lin, C. A. McDowell, J. R. Rowlands, *J. Chem. Phys.*, **35**, 757 (1961).
10. M. Ogawa, *Radiat. Phys. Chem*., **16**, 281 (1977)
11. J. Stankowski, W. Kempinski, P. ByszewskI, Z. Trybula, *Acta Phys. Pol. A.*, **88**, 1117 (1993).
12. R. Köseoğlu, E. Köseoğlu, F. Köksal, *Appl. Radiat. Isot.*, **58**, 63 (2003).
13. R. Mladenova, N. D. Yordanov, *Bulgarian Chem. Commun.*, **39**, 128 (2007).
14. K. Aleksieva, N.D. Yordanov, *Radiat. Eff. Defect. Solids*, **167**, 685 (2012).
15. U. Sayin, *J. Mol. Struct.*, **103**, 132 (2013).
16. B. Çalışkan, A.C. Çalışkan, R. Yerli, *J. Mol. Struct.*, **1075**, 12 (2014).
17. Y. Karakirova, N.D.Yordanov, *Radiat. Phys. Chem.*, **110**, 42 (2015).
18. Y. Karakirova, N. Yordanov, *Bulgarian Chem. Commun.*, **47**, 144 (2015).
19. F. Köksal, O. Çakır, I. Gümrükçü, M. Birey, *Z. Naturforsch.*, **40a**, 903 (1985).
20. N. A. Salih, A. Sanderud, E. Sagstuen, O. I. Eid, A. Lund, *J. Phys. Chem. A*, **10**, 8214 (1997).
21. M. Aydın, *Braz. J. Phys.*, **40**, 429 (2010).
22. D. J. Triggle, *Drug Develop. Res.*, **58**, 5 (2003).
23. N. Edraki, A.R. Mehdipour, M. Khoshneviszadeh, R. Miri, *Drug Discov Today***, 14**, 1058 (2009).
24. M. G. Gündüz, G. S. Öztürk, I. M. Vural, R. Şimşek, Y. Sarioğlu, C. Şafak, *Eur. J. Med. Chem.*, **43**, 562 (2008).
25. M. G. Gündüz, S. Celebi, B. Kaygısız, R. Şimşek, K. Erol, C. Şafak, *Lat. Am. J. Pharm.*, **28**, 922 (2009).
26. N. D. Yordanov, *Appl. Mag. Res*., **10**, 339 (1996).
27. M. Aydın, R. Şimşek, M.G. Gündüz, C. Şafak, Y.E.Osmanoğlu, *J. Mol. Struct.*, **1035**, 378 (2013).
28. M. H. Başkan, *Radiat. Eff. Defect. Solids*, **163**, 35 (2008).
29. K. Usta, O. O. Karakus, A. Usta, H. Deligoz, *Magn. Reson. Chem.*, **51**, 671 (2013).
30. L. Ateş, Ö. Dereli, E. Türkkan, Ü. Sayın, F. Sevgi, R. Tapramaz, M. Birey, *J. Mol. Struct.*, **1005**, 8 (2011).
31. M. H. Başkan, M. Aydın, Ş. Osmanoğlu, R. Topkaya, *Radiat. Eff. Defect. Solids*, **165**, 938 (2010).
32. D. E. Wood, R. V. Lloyd, *J. Chem. Phys.*, **53**, 3932 (1970).
33. P. Neta, R. W. Fessenden, *J. Phys. Chem.*, **75**, 738 (1971).
34. F. Köksal, Ş. Osmanoğlu, I. Kartal, F. Ucun, *Radiat. Phys. Chem.*, **49**, 537 (1997).
35. E. Bešic´, *J. Mol. Struct.*, **917**, 71 (2009).
36. M. Aydın, *Bulgarian Chem. Commun.*, **43**, 419 (2011).

СВОЙСТВА НА СВОБОДНИТЕ РАДИКАЛИ НА НЯКОИ ГАМА-ОБЛЪЧЕНИ ОРГАНИЧНИ СЪЕДИНЕНИЯ

Р. Шимшек[1], М. Г. Гюндюз[1], Дж. Шафак[1], Й. Кьокпинар[2], М. Айдън[3*]

[1]*Факултет по фармация, Университет Хаджетепе, 06100-Анкара, Турция*
[2]*Професионално училище за технически науки, Университет Адъяман, Турция*
[3]*Образователен факултет, , Университет Адъяман, Турция, 02040-Адъяман, Turkey*

Постъпила на 15 май, 2015 г.; коригирана на 3 февруари, 2016 г.

(Резюме)

Прахове на някои органични съединения (дихидро-пиридини, съединения 1-4) бяха облъчени с гама-лъчи от ^{60}Co, а спектроскопските свойства на образуваните свободни радикали бяха изследвани при обикновена температура с помощта на електронен парамагнитен резинанс (EPR). Анализът на EPR-спектрите показва, че парамагнитните продукти, получени при радиационната деструкция са $N\dot{C}H_2$. Определени са стойностите *g* на радикалите и свръх-фините структурни константи на свободните електрони с близките протони и ядрата на ^{14}N. EPR-спектрите показват, че те са на радикали, оставащи непроменени в продължение на повече от два месеца. Резултатите са в добро съгласие с тези от предишни изследвания на аминови радикали.