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Effects of gamma irradiation on the single crystal ergosterol: An EPR study

Ulku Sayin [b,*], Ömer Dereli [a], Ercan Türkkan [a]

[a] Department of Physics, Education Faculty, Selcuk University, 42090 Meram, Konya, Turkey
[b] Department of Physics, Science Faculty, Selcuk University, Konya, Turkey

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ABSTRACT

Single crystals of ergosterol were investigated by Electron Paramagnetic Resonance (EPR) technique, with γ irradiation of the crystals at different orientations in the magnetic field between temperatures of 120 and 380 K, and the spectra were found to be slightly dependent on temperature. Because of the importance of ergosterol it is important to determine the irradiation effects on this molecule.

Taking into consideration the chemical structure and the experimental spectra of the irradiated single crystal ergosterol, we found that two paramagnetic species which were labeled as radical A, $^{\cdot}CH_{2\alpha}H_{\beta}$, and radical B, $^{\cdot}CH_{\alpha}H_{\beta}H_{\gamma}H_{\sigma}$, were produced in the host crystal. The EPR parameters; spectroscopic splitting factor, *g*, and hyperfine coupling constant, a, were determined for each radical.

1. Introduction

Gamma irradiation offers definite advantages, such as the possibility of sterilization of drugs and other medical supplies and etc. in packages and it also ensures a long period of validity. Due to these advantages sterilization by gamma irradiation has been used in many countries [1,2]. Also irradiation is used for sterilization of foods and feeds [3–7]. However, it has long been known that the gamma irradiation produces radicals in the material and EPR investigations provide the most complete information about the produced radicals [8–16].

It is known that EPR spectroscopy is one of the most powerful methods for investigating molecules containing unpaired electrons. There are two EPR parameters (the A and *g* tensors) that contain valuable information about the radical systems. The A value of a given nucleus in a radical is highly sensitive to its chemical environment. In contrast to A, the *g*-tensor quantity depends on the spin distribution of the whole radical. As a consequence, it can be significantly affected by intermolecular interactions, thus providing valuable indications of the local environment of the radical [17].

Ergosterol, also called pro vitamin D_2, a white crystalline organic solid of the molecular formula $C_{28}H_{44}O$ belonging to the steroid family. It is found only in fungi (e.g., *Saccharomyces* and other yeasts) and is chemically related to cholesterol. Ergosterol is converted by ultraviolet irradiation into ergocalciferol, or vitamin D_2, a nutritional factor that promotes proper bone development in humans and other mammals [18]. Research has shown ergosterol may have anti-tumor properties [19,20].

The first analysis of EPR spectra in X-irradiated powders of some steroid hormones had been reported by Rexroad and Gordy [21]. They studied oxygen effect on the produced radicals by irradiation in steroid hormones [21] and there are some single crystal EPR studies about cholesterol type steroids [22–27]. On the other hand, to the best of our knowledge EPR investigations of gamma irradiated ergosterol single crystal have not been performed in the literature yet. In the present study, magnetic properties of gamma irradiated ergosterol single crystal were investigated experimentally using EPR technique between temperatures of 120 and 380 K.

2. Experimental

The yellow powder of ergosterol ($\geqslant$95%), the chemical structure shown in Fig. 1, was purchased from Sigma–Aldrich. The samples were crystallized in the laboratory by slow evaporation from a concentrated solution in acetone. Single crystals of 1–2 mm size were chosen and exposed to γ-rays from ^{60}Co of 0.91 kGy/h for 110 h at room temperature. About 100 kGy dose have been used for investigating of free radicals produced in cholesterol and its derivatives [22–27]. Right after irradiation, the EPR spectra of the ergosterol crystals were recorded between 120 and 380 K at 10° intervals in the magnetic field, applied along each of the three crystallographic axes (*x*, *y* and *z*) using a Bruker model EMX081 X-band EPR spectrometer. Low-and high-temperature measurements were

* Corresponding author. Tel.: +90 533 4750175; fax: +90 332 241 2499.
E-mail address: uakpinar@selcuk.edu.tr (U. Sayin).

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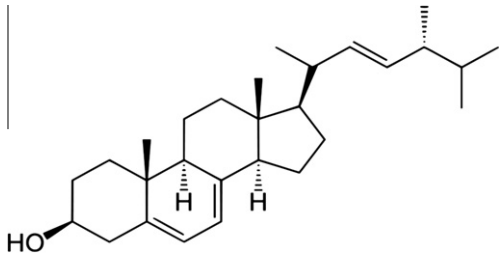

Fig. 1. Chemical structure of ergosterol.

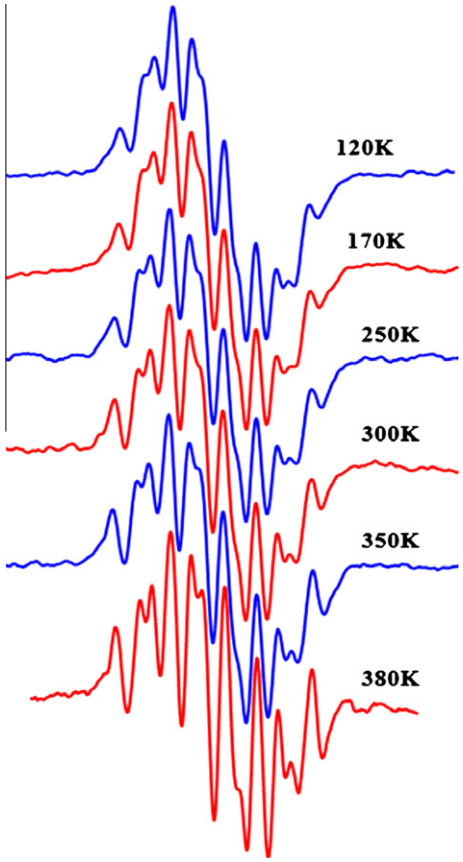

Fig. 2. The temperature dependency of EPR spectra of irradiated ergosterol single crystal.

performed using a Bruker variable temperature-control unit. The magnetic-field modulation frequency, the microwave power and the modulation amplitude of receiver were 100 kHz, 1 mW and 1 G, respectively. EPRES, the program to help for the resolution of complex single crystal EPR spectra, was used to label the peaks recorded by EPR spectrometer [28]. Bruker Simfonia software was used for spectral simulations. The *g*-factor of the radical was found by comparison with a DPPH sample ($g = 2.0036$) [29].

3. Results and discussion

Taking into consideration the spectra of ergosterol when the magnetic field oriented 0° to the *x* axis between 120 and 380 K as shown in Fig. 2, the EPR parameters of the putative radical produced by gamma irradiation were found to be slightly dependent on the temperature and as shown in Fig. 3 dependent on

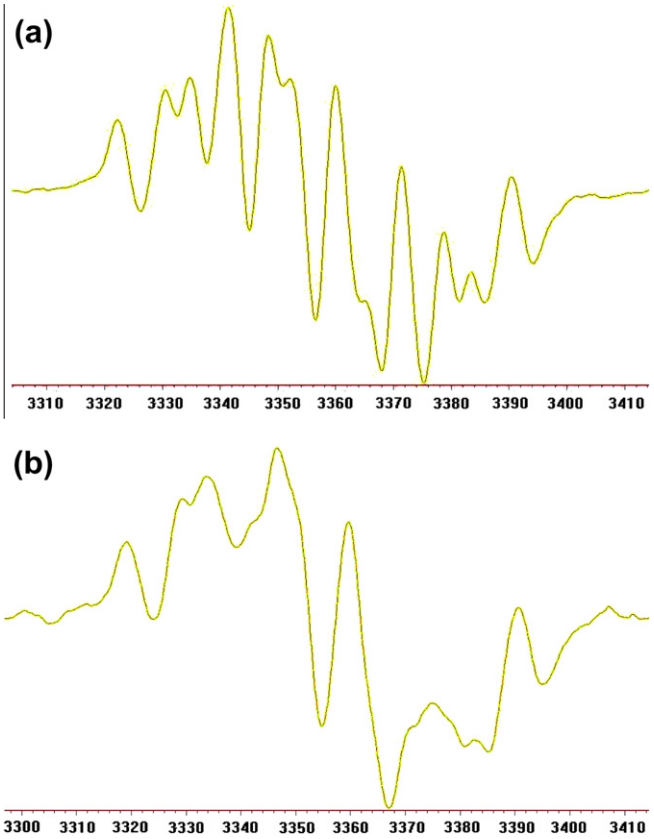

Fig. 3. The EPR spectra of irradiated ergosterol single crystal when the magnetic field oriented (a) 0° to the *x* axis, (b) 70° to the *z* axis.

the orientation of single crystal in the magnetic field. The spectra observed in the low temperature have superimposed character. Therefore the experimental values were calculated using the spectra recorded at 300 K, in different orientations. Fig. 4 shows the labeled magnetic field position of the peaks of the EPR spectra in *x*, *y*, *z* axes. The dots represent the peaks and the lines which connect the dots represent the sinusoidal fitting curves. In *x* and *y* axes there are at best 18 peaks however in *z* axis 11 peaks.

There is a little asymmetry in the recorded EPR spectra, shown in Figs. 3 and 5, of gamma irradiated ergosterol single crystal. The slightly marred symmetry in the observed curves can be possibly by the resonance of a secondary radical or by small anisotropies in the *g* factors and coupling constants [21,30]. Taking into account of the spectra recorded at each crystallographic axes 10° intervals, the labeled magnetic field positions of the peaks, the asymmetry of the spectra and the maximum observable numbers of peaks at each axes it can be said that there are two organic radicals in the host crystal. Szyczewski et al. were studied gamma irradiated 21-hydroxyprogesterone which was a derivative of cholesterol. They mentioned the slightly asymmetrical and complex EPR spectra which suggests the presence of more than one type of radicals [22].

The putative two organic radicals, expressed in Fig. 5a, were labeled as radical A, $^{\cdot}CH_{2\alpha}H_{\beta}$, and radical B, $^{\cdot}CH_{\alpha}H_{\beta}H_{\gamma}H_{\sigma}$ produced in ergosterol single crystal. The peaks of radicals A and B separated by using simulation shown in Fig. 5b. The simulation parameters for each radical are given in Table 3. The unpaired electron of radical A interact with 3 hydrogen atom that two of them are equivalent while the unpaired electron of radical B interact with 4 hydrogen atom that all of them are different from each other. Because of the nearly same environment of the unpaired electrons of each