

# ELECTRON SPIN RESONANCE OF GAMMA IRRADIATED SINGLE CRYSTAL OF ACETYLCHOLINE $\beta$ -RESORCYLATE

M. BIREY, H. AKTAŞ

Faculty of Sciences, Ankara University, 06100 Tandoğan, Ankara, Turkey

AND A. ALICILAR

Faculty of Engineering and Architecture, Gazi University  
06570 Maltepe, Ankara, Turkey

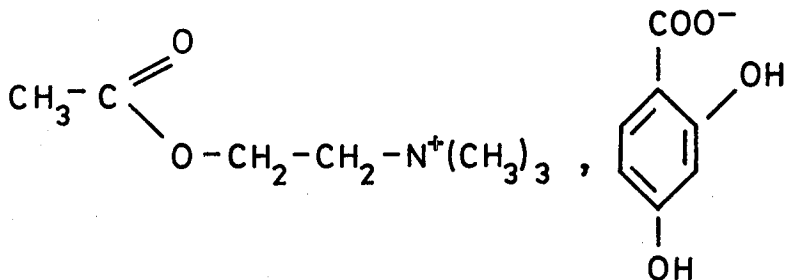
(Received December 19, 1991; in final form April 23, 1992)

The electron spin resonance of  $\gamma$ -irradiated single crystals of acetylcholine  $\beta$ -resorcyate has been observed and analyzed for different orientations of the crystals in a magnetic field.  $C_{14}H_{21}NO_6$  acetylcholine  $\beta$ -resorcyate single crystals and powders have been investigated between 120 and 360 K. The spectra were found to be isotropic down to 120 K. The isotropic value of  $g$ -factor and the hyperfine splitting constant of protons were found to be 2.0030 and 1.85 mT, respectively. The measurements indicate that 80% of unpaired electron is localized on the carbon and 20% is on the oxygens. The results are consistent with the literature data of the  $CH_3\dot{C}OO^-$  radical. PACS numbers: 33.10.+p, 35.20.Sd

## 1. Introduction

The biological importance of acetylcholine has prompted many spectroscopic studies of the conformations of acetylcholine itself and of many analogues. It was the first compound shown to function as a natural neurotransmitter [1–3]. Much work has previously been done on the electron spin resonance (ESR) spectrum, at ambient temperature, of  $\gamma$ -irradiated choline chloride whereby a predominantly 5-line spectrum was attributed to an ethanol radical,  $\dot{C}H_2CH_2OH$ ; and that assignment was confirmed by the ESR spectra of specifically deuterated choline chlorides [4]. Later the ESR spectra of choline chloride were attributed to  $(CH_3)_3N^+ \dots CH_2\dot{C}H_2OH$  biradical [5] through the more specific information obtained from the ESR study of radiation damage to single crystals and powders of choline iodide, choline sulfate,  $[(CH_3)_3NCH_2CH_2Cl]^+Cl^-$ ,

$[(\text{CH}_3)_3\text{NCH}_2\text{CH}_2\text{CH}_2\text{OH}]^+\text{Cl}^-$  and  $[(\text{C}_2\text{H}_5)_3\text{NCH}_2\text{CH}_2\text{CH}_2\text{OH}]^+\text{Cl}^-$ . The spectra of these five compounds are completely different from those of the radiation-sensitive chloride and bromide [6]. In the last years single crystals of about 8 acetylcholine itself and analogues have been irradiated with high-energy radiation and the resulting radicals were studied using the technique of ESR. It does not seem that there is any definite pattern in the bond breakage in these irradiated molecules. The present work studies, using  $^{60}\text{Co}$   $\gamma$ -rays, the influence of radiations on bond breaking in the substance acetylcholine  $\beta$ -resorcylate whose chemical formula is



## 2. Experimental

The acetylcholine  $\beta$ -resorcylate single crystals were grown in the laboratory from an aqueous solution. The acetylcholine  $\beta$ -resorcylate crystals belong to the monoclinic  $P2_1/c$  space group and the unit cell dimensions are  $a = 14.562$ ,  $b = 14.109$  and  $c = 15.096$  Å with  $\beta = 106.90^\circ$  [7]. The unit cell contains eight molecules. The single crystals and powders were irradiated at room temperature by  $^{60}\text{Co}$   $\gamma$ -ray source  $30 \text{ MGy h}^{-1}$  for 24 h. The ESR spectra were recorded with a Varian Model E-109 C ESR spectrometer using 2 mW microwave power. The low and high temperature, 120 and 360 K, were obtained using a Varian temperature control unit. The crystals were rotated on a Lucite pillar about the three axes and the angle of rotation was read on a scale graduated in degrees. The  $g$ -factor was found by comparison with a DPPH sample ( $g = 2.0036$ ).

## 3. Results and discussion

The spectra were taken at 5-deg intervals for the applied magnetic field  $H$  being in each of the three crystallographic planes  $ab$ ,  $bc$  and  $ca$ . The number and relative intensities of the hyperfine structure peaks observed in acetylcholine  $\beta$ -resorcylate suggest that these peaks result from the interaction of the unpaired electron with the protons and the spectrum consisted of four peaks of intensity ratio 1 : 3 : 3 : 1 with 1.85 mT spacing (Fig. 1) at all orientations of the magnetic field during the rotation of the crystal about its crystallographic axes and the powder spectrum is the same as in Fig. 2. These experimental data indicate that the unpaired electron spin interacts with a methyl group and therefore the spectra are attributed to  $\text{CH}_3\text{C}\ddot{\text{O}}\text{O}^-$  radicals. Since no site splittings were observed it is concluded that the 8 molecules in the unit cell are magnetically equivalent. This

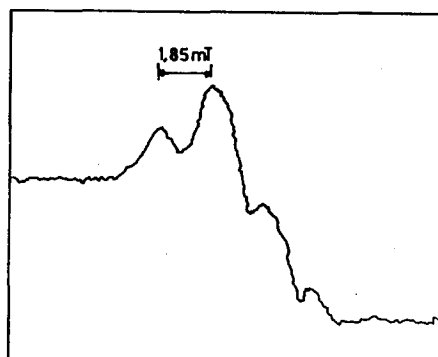
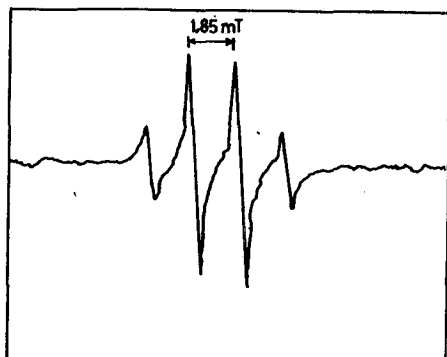


Fig. 1. Electron spin resonance spectrum of gamma irradiated acetylcholine  $\beta$ -resorcy late single crystal.

Fig. 2. Electron spin resonance spectrum of gamma irradiated acetylcholine  $\beta$ -resorcy late powder.

TABLE

Principal elements and direction cosines of the  $g$ -tensors (all  $\pm 0.0005$ ) for the  $\text{CH}_3\dot{\text{C}}\text{OO}^-$  radicals in gamma irradiated acetylcholine  $\beta$ -resorcy late.

Substance	Principal values	Direction cosines
$\text{C}_{14}\text{H}_{21}\text{NO}_6$	2.0022	-0.870 -0.069 -0.491
	2.0030	-0.466 +0.455 +0.762
	2.0038	+0.175 +0.883 -0.427
	$g_{\text{av}} = 2.0030$	

radical was observed in X-irradiated acetic acid [8]. Several radicals are known in which the presence of a methyl group dominates the appearance of the spectrum, contributing three  $\beta$ -protons to the hyperfine pattern. Rotation about  $\text{C}_\alpha\text{H}_3\text{-C}_\beta$  bond renders the three protons equivalent at room temperature, and the methyl group contributes a quartet of intensity ratio 1:3:3:1. The wide range of values of the interaction with  $\beta$ -protons has been interpreted in terms of the variation in angle ( $\theta$ ) between the  $\text{C}_\beta\text{-H}_\beta$  bond, the  $p$ -orbital direction projected perpendicularly to  $\text{C}_\alpha\text{-C}_\beta$  bond, and the  $p$  orbital which contains the unpaired electron. The  $\beta$ -proton coupling is given by [9, 10]:

$$A_\beta = B_0 + B_1 \cos^2 \theta, \quad (1)$$

where  $B_0$  is a constant and includes the contributions from spin density which arise from conformation independent mechanism, in particular spin polarization, and  $B_1$  includes the hyperconjugative contributions.

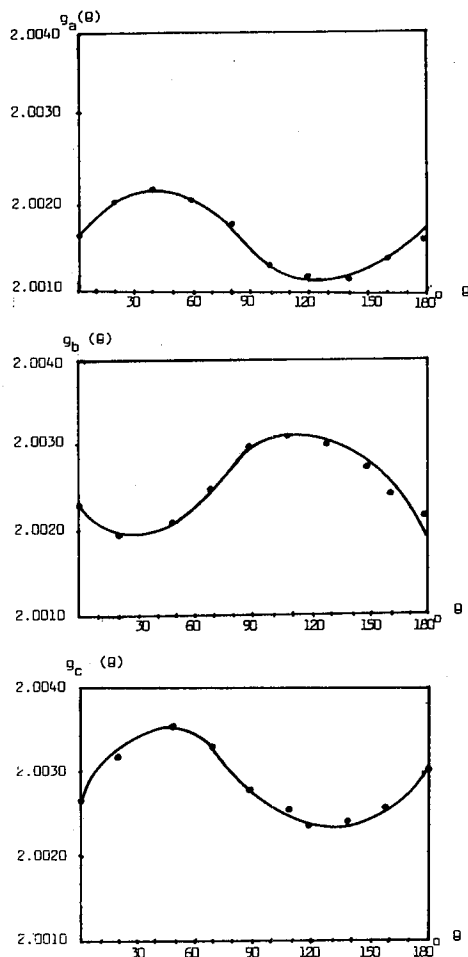


Fig. 3. Variation of the  $g$ -tensor around  $a$ ,  $b$  and  $c^*$  axes of the gamma irradiated acetylcholine  $\beta$ -resorcyate single crystal.

In the case of rapid rotation about the  $C_\alpha$ - $C_\beta$  bond the average value of  $A_\beta$  becomes

$$a_\beta = B_0 + \frac{1}{2}B_1. \quad (2)$$

In this equation  $B_0 = 0 \div 0.35$  mT [9-11] and  $B_1 = 3.21$  mT [12]. If we replace these values in Eq. (2), we obtain  $a = 1.60 \div 1.96$  mT and the value 1.85 mT obtained in this study is within this range. If  $\rho_c$  is the fraction (%) of unpaired electron density on the carbon,  $a_{\beta H}$  will be linearly proportional to it, and this is equivalent to Mc Connell's equation  $a_{\beta H} = Q\rho_c$  [16]. The hyperfine coupling of freely tumbling ( $Q$ ) methyl radical is 2.3 mT [15]. It indicates that in

$\text{CH}_3\dot{\text{C}}\ddot{\text{O}}\text{O}^-$  radical 80% of the spin density is on the carbon and 20% is on the oxygens.

This value is also in agreement with the  $\beta$ -proton coupling for  $(\text{CH}_3-\text{CH}_2)_3\dot{\text{C}}$  (1.73 mT),  $(\text{CH}_3)_2\dot{\text{C}}\text{CH}_2\text{CH}_3$  (1.76 mT) and  $>\dot{\text{C}}-\text{CH}_3$  (1.76 mT) radicals [13, 14]. The isotropic value of the  $g$ -factor is found to be 2.0030. The variation of  $g(\theta)$  is given in Fig. 3 and the principal values with their cosines are given in Table.

### References

- [1] R.M. Lemmon, M.A. Parsons, D.M. Chin, *J. Am. Chem. Soc.* **77**, 4139 (1955).
- [2] B.M. Tolbert, *J. Am. Chem. Soc.* **75**, 1867 (1953).
- [3] B. Jensen, *Aspect of the Molecular Structure of Acetylcholine and of Related Compounds*, FADL's forlag, Kobenhavn 1984.
- [4] R.O. Lindblom, R.M. Lemmon, M. Calvin, *J. Am. Chem. Soc.* **83**, 2484 (1961).
- [5] Y. Tomkiewicz, R. Agarwal, R.M. Lemmon, *J. Am. Chem. Soc.* **95**, 3144 (1973).
- [6] A. Nath, R. Agarwal, R.M. Lemmon, *J. Chem. Phys.* **61**, 1542 (1974).
- [7] B. Jensen, *Acta Chim. Scand. B* **29**, 531 (1975).
- [8] I. Miyagawsa, W. Gordy, *J. Am. Chem. Soc.* **83**, 1036 (1961).
- [9] J.R. Morton, *Chem. Rev.* **64**, 453 (1964).
- [10] W. Gordy, *The Theory and Applications of Electron Spin Resonance*, Wiley, New York 1980, p. 216.
- [11] N.M. Atherton, *Electron Spin Resonance*, Wiley, New York 1973, p. 166.
- [12] J.E. Bennet, L.H. Gale, *Trans. Faraday Soc.* **64**, 1174 (1968).
- [13] R.W. Fessenden, R.H. Schuler, *J. Chem. Phys.* **39**, 2147 (1963).
- [14] W.T. Dixon, R.O.C. Norman, A.L. Buley, *J. Chem. Soc.*, Part 3, 3625 (1964).
- [15] M.T. Rogers, L.D. Kispert, *J. Chem. Phys.* **46**, 221 (1967).
- [16] M.C.R. Symons, *J. Chem. Soc.*, Part 1, 277 (1959).