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ELECTRON SPIN RESONANCE OF AN IRRADIATED SINGLE CRYSTAL OF L-TYROSINE-HCl*

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Abstract and Summary.—Analysis of the electron spin resonance (ESR) of a γ -irradiated single crystal of L-tyrosine-HCl reveals a free radical formed by loss of an H from the hydroxyl group attached to the ring. Principal g values of the radical are $g_x = 2.0023 \pm 0.0005$, $g_y = 2.0067 \pm 0.0005$, and $g_z = 2.0045 \pm 0.0005$, with g_x perpendicular to the plane of the ring and g_y along the CO bond to the ring. Analysis of the hyperfine structure of the spectra shows electron spin density on C₍₂₎ and C₍₆₎ to be 0.24 and on C₍₄₎ to be 0.32. Corresponding spin densities calculated from molecular orbital theory are 0.26 and 0.31, respectively.

Radiation effects on various derivatives of tyrosine in the powdered or polycrystalline form have been investigated before with electron spin resonance.¹⁻³ By bombardment of the sample with H atoms, Liming and Gordy⁴ recently showed that one type of radical produced results from H addition on the tyrosine ring. However, there are other ESR signals produced by ionizing irradiation of tyrosine and its derivatives which cannot be attributed to H-addition radicals. Also, when H-addition radicals are formed by ionizing irradiation of pure tyrosine, it is evident that the added H atoms must be released from some other part of the molecule. As the possible explanation of the ESR patterns observed in γ -irradiated samples of powdered tyrosine and polytyrosine, radicals formed by loss of the hydrogen from the OH group on the ring have been postulated,^{1, 2} but this postulate could not be proved from the unresolved structure of the resonance of the powdered samples. We have undertaken this study on single crystals in the hope of correctly identifying the radicals formed and obtaining quantitative information about them.

The Tyrosine-HCl Crystal.—Single crystals of L-tyrosine-HCl were grown at room temperature by slow evaporation of L-tyrosine dissolved in 30 per cent hydrochloric acid. The dimensions of the crystal used in this study were $1 \times 1 \times 8$ mm. When the crystal was rotated about the c axis (see Fig. 1), six faces were observed. The approximate angles between these faces were measured by an optical goniometer, and the values were compared with the ones calculated from the unit cell dimensions.

X-ray diffraction studies by R. Srinivasan⁵ indicate that L-tyrosine-HCl belongs to the monoclinic system with space group $P2_1$. The unit cell dimensions are a = 11.07 Å, b = 9.03 Å, c = 5.09 Å, with $\beta = 91.8^{\circ}$. There are two molecules per unit cell with the point transformation from I to II being $x \to -x$, $y \to y + \frac{1}{2}$, and $z \to -z.^6$ As the crystal is slightly hygroscopic, care must be taken to keep it dry. Figure 1 illustrates the form of the L-tyrosine crystal and the set of orthogonal axes chosen. The numbering system used in this paper is that of Srinivasan.

Experimental Procedure.—The crystal was subjected to a γ -ray dosage of about 9.6 million R from a cobalt-60 source having a strength of 10⁵ R/hr. Upon irradiation, the crystal changed from a nearly colorless state to a pale yellowish-brown. Irradiation and all observations were made at room temperature with ESR spectrometers at both X band (9 Gc/sec) and K band (25 Gc/sec). Spectra were recorded at intervals of 10° or less.

Spectral Observations.—Immediately after irradiation, ESR spectra of L-tyrosine-HCl were recorded with the magnetic field at various orientations in the bc', ac', and ab planes of the crystal. The spectral pattern was found to vary little with angle, the dominant feature being the five center lines which were spread over 25–33 gauss according to the orientation. The intensity distribution of the five lines was predominantly 1:2:1:2:1, changing to 1:2:2:2:1 for certain orientations. Two other quite anisotropic lines with a splitting of from 45 to 65 gauss were observed, one on each side of the central five; these two lines disappeared after a few days. The unstable radical producing them has not yet been identified.

The intensity ratio and splitting of the five lines suggest an isotropic β -proton doublet with each component further split by two nearly equivalent α -protons having anisotropic coupling (see Fig. 2). These facts and the principal directions of the g factor, which indicated that the electron spin density was distributed in the ring, suggested a radical formed by loss of an H atom from the hydroxyl group attached to the tyrosine. The hyperfine structure can be explained upon assumption that this radical is a hybrid of the four structures:



with spin density located on $C_{(2)}$, $C_{(6)}$, $C_{(4)}$, $O_{(1)}$. A quantitative molecular orbital treatment of the radical is given in a later section. One would expect anisotropic coupling to the protons $H_{(2)}$ and $H_{(6)}$, and isotropic coupling by hyperconjugation to $H_{(4)}$ and $H_{(7)}$. From the X-ray diffraction data,⁵ $H_{(4)}$ is found to be nearly in the plane of the ring; hence we observe no coupling to it. Experimentally, the isotropic hyperfine splitting at $H_{(7)}$ was measured to be 14.0 \pm 0.5 gauss. The hyperfine splitting of $H_{(2)}$ and $H_{(6)}$ tended to be nearly equivalent and varied experimentally with orientation from 5.5 gauss to 9 gauss.

FIG. 1.—Diagram of a single crystal of L-tyrosine-HCl showing the choice of reference axes. a and b are crystal axes with c'chosen to form an orthogonal set. The angle between the crystal axis c and reference axis c' is less than 2°.

Principal Elements of the g and A Tensors.—The g tensor was computed from a least-squares fitting of the general formula⁷

$$g^2 = k_1 + k_2 \cos 2\theta + k_3 \sin 2\theta \tag{1}$$

to the data, where k_1 , k_2 , and k_3 are the parameters to be determined. The g factor of the center of the spectrum was calculated with respect to 1,1-diphenyl-2-picrylhydrazine (DPPH) and Mn⁺⁺ in MgO, which were used as markers, by use of the relation

$$g \simeq g_{\rm DPPH} + \frac{2({\rm H}_{\rm DPPH} - {\rm H})}{{\rm H}_{\rm DPPH}} \,. \tag{2}$$

This relation can be derived from the resonance condition $h\nu = g\beta H$, where $g_{\text{DPPH}} = 2.0036$ and where H_{DPPH} is the field at which the 1,1-diphenyl-2-picryl-hydrazine line occurs, i.e., near 3000 gauss for X band.

The variation in g factor is seen from Figure 3 to be symmetric about the crystal axis in the ab and bc' planes, as is expected from the crystal symmetry with two magnetically distinct molecules per unit cell.



FIG. 2.—Observed spectra for two different orientations of the crystal in the magnetic field. The 14-gauss splitting is the isotropic β -proton doublet which is further split by the two nearly equivalent α -protons having anisotropic couplings. The two patterns show approximately the maximum and minimum α -proton couplings that were measured directly. The arrow above the lines in each spectrum indicates the position where the DPPH resonance (g = 2.0036) would occur.





FIG. 3.—Calculated and observed g-factor variation for the two radicals in the unit cell (I and II) for rotation of the crystal about the a, b, and c' axes with the magnetic field in the bc', ac', and ab planes, respectively. Only spectra from one radical at a time were prominent.

The radicals with the two different orientations, henceforth called (I) and (II), are equivalent along the crystal axes, and remain equivalent when the crystal is rotated about the *b* axis with the magnetic field in the *ac'* plane. The largest *g* value is observed when the magnetic field is in the *ac'* plane 60° from the *c'* axis. According to X-ray diffraction data, the direction of largest *g* corresponds to the $O_{(1)} C_{(1)} C_{(4)}$ direction, which happens to be nearly the same for radicals (I) and (II).

From the g variation in the bc' plane, where the smallest g value is observed, the g value is practically the same, within experimental error, along the c' and baxes. This indicates that the minimum g, which corresponds to the normal to the ring, must form approximately 45° angles to the c' and b axes. It was also assumed that, in the ac' plane, radical (II) produced the predominant spectra from 0° to 90° whereas radical (I) produced the predominant spectra from 90° to 180°.

The principal elements of the g tensor and their direction cosines as listed in Table 1 were found from diagonalization of the g tensor.

By a similar procedure the principal elements of the anisotropic couplings of the two equivalent protons $H_{(2)}$ and $H_{(6)}$ were found, together with their direction cosines. The resulting values are listed in Table 2 with those of the isotropic coupling protons $H_{(4)}$ and $H_{(7)}$.

Experimental and Theoretical Spin Densities.—From the isotropic component a_s in the $H_{(2)}$ and $H_{(6)}$ coupling the spin densities in the p_x orbital of $C_{(2)}$ and $C_{(6)}$ can be found from the McConnell relation

$$a_s(\mathbf{H}) = \rho_{\mathbf{C}} Q, \qquad (3)$$

where Q has the value of -26 gauss.⁸ From the experimental coupling in Table 2,

TABLE 1. Principal elements of the g tensor.

		\sim Direction Cosines for Radicals (I) and (II) \sim \sim		
		in abc' System		
Value	Direction	X-ray diffraction	ESR	
$g_x = 2.0023 \pm 0.0005$	\perp to ring	$(\pm 0.36, 0.70, \pm 0.62)$	$(\pm 0.35, 0.57, \pm 0.74)$	
$g_y = 2.0067 \pm 0.0005$	Along CO bond	$(\pm 0.84, 0.04, \pm 0.54)$	$(\pm 0.89, -0.06, \pm 0.46)$	
$g_z = 2.0045 \pm 0.0005$	\perp to g_x and g_y	$(\pm 0.39, 0.72, \mp 0.57)$	$(\pm 0.31, 0.82, \pm 0.49)$	

TABLE 2. Principal elements of the proton coupling and their directions.

Coupling atom	Coupling element* in gauss	Direction
H ₍₂₎ or H ₍₆₎	$a_x = -6.5 \pm 0.5$	\perp to plane of ring
	$a_y = -9.0 \pm 0.5$	In plane of ring and \perp to CH bond
	$a_z = -3.1 \pm 0.5$	to CH bond
H ₍₇₎	14.0 ± 0.5	Isotropic
$H_{(3)}, H_{(5)}, and H_{(4)}$	~ 0	

* The sign of the coupling is determined by theoretical calculation. See McConnell, H. M., and J. Strathdee, *Mol. Phys.*, 2, 129 (1959).

 $a_s = -(1/3)(9.0 + 6.5 + 3.1) = -6.2$ gauss for either H₍₂₎ or H₍₆₎. Thus the spin density on C₍₂₎ or on C₍₆₎ is 0.24.

The spin density on $C_{(4)}$ can be estimated from the isotropic coupling on $H_{(7)}$ from the relation

$$a_{\beta} = A_0 \rho_{\alpha} \cos^2 \theta, \tag{4}$$

where θ is the angle between the C₍₄₎-C₍₇₎-H₍₇₎ plane and the p_x orbital on C₍₄₎ and where $A_0 = 58$ gauss.⁸ From the structure of the undamaged crystal, the value of θ is about 30° for $H_{(7)}$, which has an observed isotropic coupling of 14 gauss. Thus $\dot{\rho}_{\alpha}$ for C₍₄₎ is found from equation (4) to be 0.32. From this spin density and the structural value of $\theta_{H_{(4)}} = 79^\circ$, one finds the splitting by H₍₄₎ to be 0.7 gauss. This is too small to be resolvable, which is in agreement with the observation.

Theoretical values of the spin densities have been calculated with the Hückel molecular-orbital (MO) theory⁹ and with the McLachlan self-consistent field (SCF) theory.¹⁰ As with semiquinone radicals studied by Vincow and Fraenkel,¹¹ the spin densities calculated with MO theory are quite dependent on the heteroatom parameters chosen for oxygen.

Heteroatoms are treated in MO theory by use of the following corrections to the coulomb and resonance integrals:

$$\alpha_x = \alpha + h_x \beta,$$
$$\beta_{cx} = k_{cx} \beta.$$

where α and β refer to carbon-carbon bonds. For oxygen bonds contributing one π electron, i.e., double bonding, h_0 is near unity. For single bonds where two π electrons are contributed, h_0 is about 2. Typical values are given by Pullman and Pullman¹² and by Streitwieser.¹³ The Pullman values for a C=O bond, $h_0 = 1.2$ and $k_{\rm CO} = 2$, gave reasonable results; but the values $h_0 = 1.2$ and $k_{\rm CO} =$ 1.56 as found by Vincow and Fraenkel¹¹ to give the best fit for semiquinones were used. The spin densities calculated from Hückel's theory were too small. The SCF theory predicted values in better agreement with experimental results. Comparisons are made in Table 3.

Conclusions.—Two different types of free radicals formed by the tyrosine group have now been conclusively identified. One of these, radical (E) identified earlier by H bombardment,⁴ is formed by H addition on $C_{(2)}$ or $C_{(6)}$; the other

Atom	Experimental			
	values	SCF theory	Hückel theory	
$C_{(1)}$	~ 0	-0.03	0.07	
C(2)	0.24	0.26	0.20	
C(3)	~ 0	-0.07	0.01	
C ₍₄₎	0.32	0.31	0.24	
C(5)	~ 0	-0.02	0.01	
C(6)	0.24	0.26	0.20	
O ₍₁₎		0.28	0.26	

TABLE 3. Spin densities.

radical (F), identified in the present work, is formed by H abstraction. In



both these radicals the unpaired electron is located in a π orbital of the ring. Both radicals are evidently produced by ionizing irradiation of powdered samples,^{1, 2} but in the present single-crystal experiment the H-addition radical was not observed. Stearic factors caused by the ordered structure of the crystal or the presence of HCl probably prevented observation of radical (E). The relative degree of production of the two types of radicals by irradiation of a biochemical system is expected to depend not only upon the physical condition (temperature, viscosity, etc.,) but upon the chemical composition of the over-all sample, particularly upon the acid-base balance. What has been proved is that both radicals are formed and can be stabilized in solids. The tyrosine group can give as well as take an H atom. Probably this dual capacity is of great significance in its biological functions.

Abbreviations used: ESR, electron spin resonance; MO theory, molecular-orbital theory; SCF theory, self-consistent field theory; DPPH, 1,1-diphenyl-2-picrylhydrazine.

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