Reprinted from JOURNAL OF MOLECULAR SPECTROSCOPY, Volume 9, No. 6, December, 1962
 Printed in U.S.A.

JOURNAL OF MOLECULAR SPECTROSCOPY **9**, 429–435 (1962)

Electron Spin Resonance of Gamma-Irradiated Single Crystals of Acetyl-*d,l*-Alanine and Chloroacetyl-*d,l*-Alanine*

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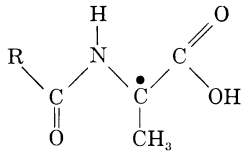
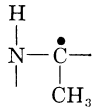
The electron spin resonance spectra of gamma-irradiated single crystals of acetyl-*d,l*-alanine and chloroacetyl-*d,l*-alanine have been measured at 9 kMc/sec and 23.4 kMc/sec for various orientations in the external magnetic field. From the analysis of the hyperfine interaction constants, the free radical produced by gamma irradiation in both compounds is found to be:



The electron spin densities at the carbon atom of the



group is approximately 0.76 and 0.86 for acetyl-*d,l*-alanine and chloroacetyl-*d,l*-alanine, respectively.

INTRODUCTION

The present article is part of a program of this laboratory directed toward the understanding of radiation damage to proteins. The electron spin resonance spectra of gamma-irradiated acetyl-*d,l*-alanine and choloroacetyl-*d,l*-alanine have been observed in the polycrystalline state (*1*). Although much valuable information has been obtained by these observations, a complete analysis demands the use of single crystals.

This article is concerned with the electron spin resonance analysis of the free

* This research was supported by the U. S. Air Force through the Air Force of Scientific Research, Air Research and Development Command.

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radicals produced by gamma-irradiation of single crystals of acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine. The structure of the free radical formed in acetyl-*d*,*l*-alanine as determined in this experiment is the same as that reported for this compound by Box, Freund, and Lilga (*2*).

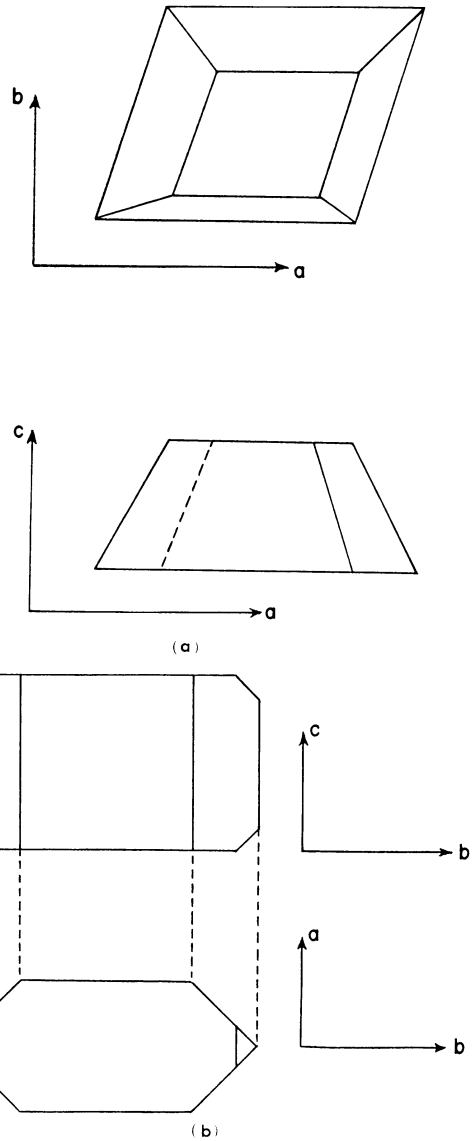


FIG. 1. Crystal forms and the coordinate axis employed. (a) Acetyl-*d*,*l*-alanine; (b) chloroacetyl-*d*,*l*-alanine.

EXPERIMENTAL

Single crystals of acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine were grown by slow evaporation from aqueous solutions of these compounds. The crystal forms of these compounds are shown in Figs. 1(a) and (b). The crystal of acetyl-*d*,*l*-alanine is known to be rhombic (*3*), but the crystal symmetry of choloracetyl-*d*,*l*-alanine is unknown. The coordinate axes *a*, *b*, and *c* shown in Fig. 1 are chosen as a convenient reference system for the electron spin resonance measurements. The cleavages are complete in the planes perpendicular to the *c* axes in acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine.

The single crystals were irradiated by the gamma rays from a kilocurie cobalt-60 source at room temperature. The normally colorless crystals acquire a pale yellow color following exposure to the gamma radiation. Electron spin resonance absorption spectra were taken at room temperature for various orientations of the crystals in the static magnetic field at 9 kMc/sec and 23.4 kMc/sec.

The *g* values shown in Table I are obtained by comparing the spectra with the location of the DPPH marker at $g_{\mathrm{DPPH}} = 2.0036$. As shown in the table, the values of the *g* factor for the two compounds are nearly isotropic and very nearly that of the free spin value, $g_s = 2.0023$.

The structural pattern of the electron spin resonance pattern of irradiated chloroacetyl-*d*,*l*-alanine does not change with time. However, the electron spin resonance pattern of acetyl-*d*,*l*-alanine undergoes several marked changes before assuming structural stability. The measurements reported in this paper for acetyl-*d*,*l*-alanine refer to the stable free radical, observed several days following the termination of the radiation.

Figure 2 shows typical electron spin resonance absorption spectra of the stable free radicals observed at 9 kMc/sec in acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine. Similar second derivative tracings of the true absorption spectra were obtained at 23.4 kMc/sec. For the spectra shown in Fig. 2, the static magnetic field was applied along the *c* axis. For this direction, all of the acetyl-*d*,*l*-alanine molecules in the unit cell are magnetically equivalent. The crystal symmetry of chloroacetyl-*d*,*l*-alanine is unknown. It is uncertain therefore whether Fig. 2(b) shows the spectrum of magnetically one kind of a free radical.

Measurements of the crystal for various orientations of the crystal with respect to the magnetic field at both microwave frequencies show, however, that both the *g* factor and the hyperfine coupling constant to be isotropic. Hence the mag-

TABLE I

THE *g*-FACTORS IN DIFFERENT DIRECTIONS

Direction of magnetic field	Acetyl-*d*,*l*-alanine	Chloroacetyl-*d*,*l*-alanine
a	2.0032	2.0030
b	2.0039	2.0050
c	2.0028	2.0036

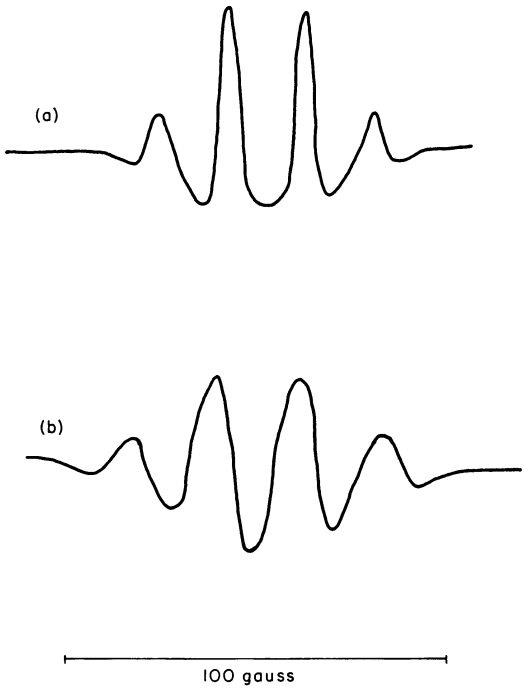


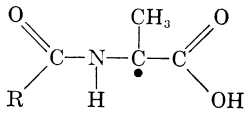
FIG. 2. Electron spin resonance spectra measured at 9 kMc/sec. The magnetic field along the c-axis. (a) Acetyl-*d*,*l*-alanine; (b) chloroacetyl-*d*,*l*-alanine.

netically different free radicals give the same spectra. It is the reason that Fig. 2(b) is considered to represent the spectrum of the magnetically one kind of the free radical.

NATURE OF THE FREE RADICALS

The spectra of the free radicals in chloroacetyl-*d*,*l*-alanine and acetyl-*d*,*l*-alanine consist of four hyperfine lines of equal spacing, with components in the intensity ratio 1:3:3:1. The spacing of the component lines is nearly isotropic, 19 gauss in acetyl-*d*,*l*-alanine and 21.5 gauss in chloroacetyl-*d*,*l*-alanine. The measurement for acetyl-*d*,*l*-alanine agrees approximately with that of 19 gauss as reported by Box *et al.* for the polycrystalline sample.

The observed spectra are characteristic of an equal coupling of an unpaired electron with three hydrogen nuclei in the group $=\dot{C}-CH_3$. Therefore the free radicals are of the form



where R represents the CH_3 group in acetyl-*d*,*l*-alanine and the CH_2Cl group in chloroacetyl-*d*,*l*-alanine.

The hyperfine interaction of a π-proton with an unpaired electron density ρ_C on the neighboring unsaturated carbon atom has been determined experimentally and theoretically (*4*) to be,

$$a = Q\rho_C , \tag{1}$$

where the value of Q is 25 gauss. Therefore measured hyperfine couplings of 19 and 21.5 gauss lead to values of 0.76 and 0.86 for the spin densities at the carbon atom in acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine, respectively.

The interaction between the proton of the NH group and the π-electron on the carbon would yield an isotropic coupling expressed as

$$b = 2Q' \cos^2 \theta \rho_C . \tag{2}$$

ρ_C is the spin density at the carbon atom and θ is the angle between the projections of the NH bond and the unpaired π-orbital in the plane perpendicular to the NC bond. Since the proton in the NH group is usually in the plane of the molecular skeleton, θ in Eq. (2) is 90°. Thus no isotropic interaction is expected between the NH group proton and the unpaired electron.

The line width of the electron spin resonance spectra of these free radicals was analyzed in the same manner as for *N*-acetyl glycine (*5*) and glycyl-glycine (*6*). A large contribution of the line broadening may be attributed to the interaction between the unpaired electron spin and the N^{14} nuclear magnetic moment and the unpaired electron density at the carbon atom as well as with the unpaired "contact" spin density with the nuclear moment.

The dipole dipole interaction may be determined by assuming the calculated spin density ρ_C to be centered at the carbon atom. Under these conditions the N^{14} nucleus gives rise to an effective field H_i acting on ρ_C, which in the strong field case is expressed by

$$H_i = (M_I{}^N/I^N)\mu_I{}^N\beta_I\langle 1/r^3\rangle \text{ av}(\cos^2 \theta - 1). \tag{3}$$

The maximum values of the effective field are 1.9 gauss and 2.2 gauss along the CN bond for $\rho_C = 0.76$ and $\rho_C = 0.86$, respectively, using $R_{CN} = 1.48$A. At right angles to the bond the coupling would be -0.9 gauss and -1.1 gauss for acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine.

Another contribution to the width of the absorption line is spin density at the nitrogen nucleus. The hyperfine components due to spin density at the nitrogen nucleus is expressed by the hyperfine constant,

$$A_2{}^N = l^2\rho_N A_f + m^2\rho_N A_\mu(3 \cos^2 \theta - 1), \tag{4}$$

where l^2 and m^2 are *s*- and *p*-characters of the unpaired electron. The spin density at nitrogen atom arises from a spin polarization of σN—C bond through configuration interaction on the carbon. The unpaired spin on the N atom is ap-

TABLE II

N^{14} Coupling Constant in Acetyl-*d*,*l*-Alanine and Chloroacetyl-*d*,*l*-Alanine

	Acetyl-*d*,*l*-alanine		Chloroacetyl-*d*,*l*-alanine	
	Observed	Calculated	Observed	Calculated
Maximum value perpendicular to C—N bond	5 gauss	−5.8 gauss	6 gauss	−6.8 gauss
Minimum value along C—N bond	3.5	−3.9	5	−4.4
ρ_N (assumed)		3%		3.5%

parently a hybrid one and we can assume it to be sp^2 hybrid. If the values of $A_f = 520$ gauss and $A_\mu = 14$ gauss as calculated by Smith, Sorokin, Gelles, and Lasher (*7*) in the analysis of ESR of nitrogen donors in diamond are used,

$$A_2{}^N = 192\rho_N \text{ gauss along the CN bond}$$

and

$$A_2{}^N = 163\rho_N \text{ gauss perpendicular to the CN bond.}$$

These calculated values may lead to a major contribution to the width of the spectral components lines.

The splitting due to the nitrogen nucleus was not resolved in either acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine. However, the order of their magnitude may be estimated from the absorption line width. The measured maximum and minimum values of this coupling are shown in Table II. The calculated coupling constants are also listed in this table, which includes the contributions of H_i in Eq. (3) and of $A_2{}^N$ in Eq. (4). ρ_N are assumed to be −0.03 and −0.035 in acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine, respectively. The satisfactory agreement between observed and calculated coupling constants are obtained. When the values of $A_f = 273$ gauss and $A = 13.7$ gauss are used as determined by Gordy and Miyagawa in the analysis of dimethylglyoxime (*8*), the satisfactory agreement between the observed and calculated values were obtained, if we assume ρ_N as −0.05 and −0.06 in acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine, respectively. Good agreement was not obtained if the positive spin density at the N atom was assumed. Thus the negative spin density at the N atom is confirmed in the free radical produced from acetyl-*d*,*l*-alanine and chloroacetyl-*d*,*l*-alanine. It is also shown that the negative spin density at the N atom is proportional to the spin density at the neighboring carbon atom.

Acknowledgment

The author is grateful to the United States Educational Foundation in Japan for awarding him the Fulbright travel grant. He is deeply indebted to Professor Walter Gordy and Dr. Robert Lontz for valuable discussions and suggestions.

Received March 16, 1962

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