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ELECTRON SPIN RESONANCE STUDIES OF IRRADIATED SINGLE CRYSTALS OF DIHYDROTHYMINE*

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July 1969

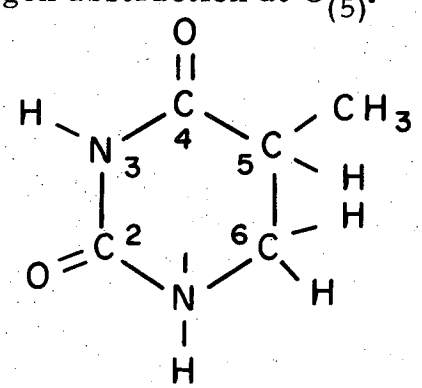
ABSTRACT

Both the 5-thymyl radical and the 6-thymyl radical are formed when dihydrothymine is irradiated at room temperature. In this paper the 6-thymyl radical is analyzed in detail by use of electron spin resonance (ESR) spectroscopy. Principal values for the spectroscopic splitting tensor are $g_u = 2.0039$, $g_v = 2.0031$, and $g_w = 2.0022$. Hyperfine coupling to the β proton is nearly isotropic, with $A^{\beta} = 44.0$ gauss. The α-proton hyperfine tensor has principal values, in gauss, of $A_x^{\alpha} = 7.8$, $A_y^{\alpha} = 26.9$, $A_z^{\alpha} = 17.5$. Comparisons between the principal axes for the ESR parameters and the known crystal structure of dihydrothymine indicate that radical formation is accompanied by a conformational change in the pyrimidine ring. This change results in a radical structure which is more nearly planar than the original puckered dihydrothymine molecule. Irradiation and observation at 77°K revealed that both the 5-thymyl radical and the 6-thymyl radical are formed, but the relative quantities of the two radicals are different than at room temperature.

Short title: ESR of Irradiated Dihydrothymine

INTRODUCTION

Several electron spin resonance (ESR) studies have shown that the 5-thymyl radical is formed when compounds containing the thymine ring are exposed to ionizing radiation[1-3] or thermal hydrogen atoms.[4, 5] The hydrogen addition at $C_{(6)}$ breaks the 5, 6 double bond, leaving an unpaired electron localized mainly in a $2p\pi$ orbital on $C_{(5)}$ of the pyrimidine ring. The 5-thymyl radical is also formed in dihydrothymine (I) by a hydrogen abstraction at $C_{(5)}$.[6-9] In this compound, it has been



(I)

reported that the 6-thymyl radical is also formed, both by ionizing radiation[7, 8] and by exposure to hydrogen atoms.[6] Identification of the 6-thymyl radical was not unambiguous, however, since polycrystalline samples were used in those studies and large anisotropies are to be expected in the ESR spectra for this radical. We have now obtained single crystals of dihydrothymine and have carried out ESR studies which confirm the presence of the 6-thymyl radical in this compound after irradiation. The parameters characterizing the ESR spectra of the 6-thymyl radical in dihydrothymine are analyzed in this paper, and some information is presented about changes in molecular conformation accompanying radical formation.

EXPERIMENTAL PROCEDURE

Polycrystalline dihydrothymine was obtained commercially and used without further purification. Single crystals were grown by slowly cooling an aqueous solution saturated at 80°C. The crystals used for ESR studies were quite small, weighing typically about 0.8 mg, but had a well-developed crystal habit. It was possible, from the features described by Furberg and Jensen,[10] to determine the crystallographic axes in relation to the crystal morphology. The crystals are orthorhombic, space group Pbca, with eight molecules in the unit cell. The unit cell parameters, in angstroms, are a = 7.336, b = 23.474, c = 7.034. A more detailed description of the molecular structure and arrangement within the crystals is given later, in connection with the ESR analysis.

The crystals were irradiated with 6.5-MeV electrons from a linear accelerator. Total exposures of 25 to 50 MR were delivered at a rate of 1 MR/min. The exposure temperature was either 295°K or 77°K. In the experiments at 77°K the crystal was irradiated in one end of a quartz tube with the other end of the tube shielded against radiation. After exposure the crystal could be transferred, without warming, to the shielded end, which was made to hold the crystal in a desired orientation.

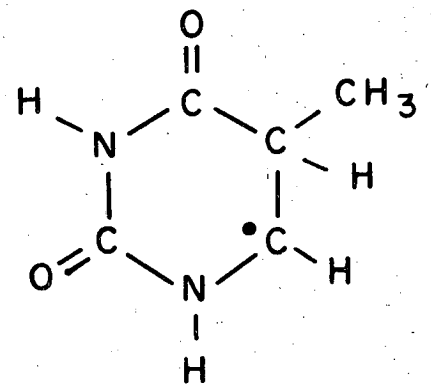
ESR spectra were obtained with a Varian 4502 spectrometer. The spectra recorded at room temperature were obtained at 16 GHz with a microwave bridge constructed in our Laboratory, whereas the low-temperature spectra were taken with the Varian X-band rotating cavity. Hyperfine splittings were measured by comparison with the known splittings of a sample of Mn^{++} in MgO. DPPH was used as a standard for g-value measurements.

The calculations necessary for determining spin resonance parameters were made by using a CDC 6600 computer.

ANALYSIS OF SPECTRA

Two radicals are formed in significant quantities when dihydrothymine is irradiated at room temperature. The hyperfine spectrum of the 5-thymyl radical, with a total splitting of about 135 gauss, is easily recognizable in the spectrum of Fig. 1, where arrows indicate its outer components. Radicals of this type have been studied previously in other compounds, and no thorough analysis of the 5-thymyl radical in irradiated dihydrothymine was made.

The other radical present in irradiated dihydrothymine has a hyperfine spectrum consisting of four lines of approximately equal intensity. Bars beneath the spectra of Figs. 1 and 2 indicate this quartet pattern. The hyperfine splitting is due to unequal coupling of the unpaired electron to two protons. At various crystal orientations, the coupling to one proton remained constant at 44.0 gauss, whereas the other proton coupling varied from about 10 to 26 gauss. The g value was slightly anisotropic, with values ranging from 2.0022 to 2.0038. This behavior is typical for a radical with the unpaired electron in a carbon atom's $2p\pi$ orbital, with hyperfine coupling to one α proton and one β proton. In the absence of drastic molecular rearrangement, the only radical that can be formed in dihydrothymine with these properties is the 6-thymyl radical (II).



(II)

The anisotropies in the g tensor and the α-proton hyperfine tensor can be used, along with the known crystal and molecular structure,[10] to confirm this assignment for the radical giving rise to the quartet spectrum.

DETERMINATION OF SPIN RESONANCE PARAMETERS

The resonance lines observed can be adequately described by a spin Hamiltonian of the form

$$\mathcal{H} = \beta \vec{S} \cdot \underset{\sim}{g} \cdot \vec{H} + \vec{S} \cdot \underset{\sim}{A}^{\alpha} \cdot \vec{I}^{\alpha} + A^{\beta} S_z I_z^{\beta},$$

where $\underset{\sim}{g}$ is the spectroscopic splitting tensor, $\underset{\sim}{A}^{\alpha}$ is the α-proton hyperfine tensor, and A^{β} is the isotropic β-proton hyperfine coupling. The external magnetic field $\vec{H}$ is assumed to be in the z direction, and β, $\vec{S}$, and $\vec{I}$ are the Bohr magneton, the electron spin operator, and the nuclear spin operator, respectively. Determination of the principal values of $\underset{\sim}{g}$ and $\underset{\sim}{A}^{\alpha}$ by the method of Schonland[11] requires measurement of the maximum and minimum values of these parameters in three planes. This was possible, with some difficulty, for the three crystallographic planes in dihydrothymine. Although there are

eight molecules per unit cell in the dihydrothymine crystal, our ESR observations show that the radicals occupy only two magnetically distinct sites in the unit cell. Table I gives the principal values for $\underset{\sim}{g}$ and $\underset{\sim}{A}^{\alpha}$, along with the corresponding direction cosines relating the principal axes to the crystallographic abc axis system for one molecule in the unit cell. The observed and calculated values for the spin resonance parameters are compared in Figs. 3 and 4 for the ab and ca planes. Although the maximum and minimum values of g and A^{α} could be determined in the bc plane, it was not possible to measure the values at all orientations, due to overlapping lines and interference from the spectrum of the 5-thymyl radical.

RADICAL ORIENTATION AND CONFORMATION

The crystal structure of dihydrothymine is an extremely complicated one. The dihydrothymine molecule itself is not planar, so that both D and L molecules exist. According to Furberg and Jensen,[10] both enantiomorphs occupy all sites in the crystal structure. The two enantiomorphs do not occur in equal quantities, however; the ratio of D molecules to L molecules is 3:2. The structure is therefore disordered, in the sense that the two forms occupy all sites randomly, but with unequal probability.

With the exception of $C_{(5)}$ and $C_{(6)}$, all the nonhydrogen atoms of the dihydrothymine molecule lie nearly in the same plane. Thus, the planar portion of the structure is presumably ordered. Our ESR measurements indicate order rather than disorder with regard to the site occupancy of the observed radicals. The maximum number of

magnetically distinct sites observed is two, whereas the disordered state should give four, these constituting two unequally weighted pairs. There are two possible ways for this to occur. First, if radicals were formed on only the D or the L molecules, the disorder of the crystal structure would not be reflected in the magnetic symmetry of the ESR measurements. Second, if the radical were formed in such a way that the α-carbon was part of the planar structure, the radical symmetry would show order rather than disorder. A combination of the two possibilities cannot, of course, be ruled out.

The above possibilities can be examined more closely by using information available from the ESR analysis. On theoretical grounds, the directions of g_w and A_z^α are along the p orbital containing the unpaired electron.[12, 13] For the 6-thymyl radical, this direction is perpendicular to the plane formed by $N_{(1)}$, $C_{(6)}$ and $C_{(5)}$. Since the atomic coordinates for the atoms in the parent molecule are known,[10] the direction perpendicular to this plane can be calculated for both D and L molecules, assuming no conformational changes. For both enantiomorphs, these calculations show relatively poor correlation between this direction and the directions for g_w and A_z^α given in Table I.

Much better agreement with the ESR results are obtained if it is assumed that the parent molecule is changed in such a way that $C_{(6)}$ becomes a part of the planar structure. In this case the p orbital of the unpaired electron would be perpendicular to the plane of the atoms $N_{(1)}$, $C_{(2)}$, $N_{(3)}$, and $C_{(4)}$. The calculations show that g_w and A_z^α are only 16 deg and 10 deg, respectively, from this direction.

It seems likely, therefore, that the conformation of the newly formed 6-thymyl radical changes to accommodate sp^2 hybridization at $C_{(6)}$, so that $C_{(6)}$ becomes almost part of the ordered structure. Likewise, $C_{(5)}$ also probably undergoes some rearrangement, although the entire ring structure of the radical may not necessarily be perfectly planar.

Additional evidence for this model for the orientation and conformation of the 6-thymyl radical comes from the direction of A_x^{α}. The minimum hyperfine coupling to an α proton should occur in the direction of the $C\text{-}H^{\alpha}$ bond.[13] On the assumption that $C_{(6)}$ becomes coplanar with $N_{(1)}$, $C_{(2)}$, and $N_{(3)}$, the direction of the $C_{(6)}\text{-}H^{\alpha}$ bond should be very near the direction of the $C_{(2)}\text{-}N_{(1)}$ bond. Calculations based on the atomic coordinates[10] show that the direction of A_x^{α} is only 11 deg from the direction of the $C_{(2)}\text{-}N_{(1)}$ bond.

It seems, therefore, that the radicals appear ordered because the α carbon is part of the planar, ordered structure, even though $C_{(6)}$ is disordered in the parent compound. It cannot be determined, however, whether radical formation occurs randomly on both D and L molecules or whether one enanthiomorph is favored.

LOW-TEMPERATURE STUDIES

Irradiation and observation at 77°K revealed that both the 5-thymyl and 6-thymyl radicals are formed in dihydrothymine (Fig. 5). At this temperature, the concentration of the 5-thymyl radical relative to that of the 6-thymyl radical is less than at room temperature. When samples irradiated at 77°K are warmed to room temperature, both the relative and absolute amounts of the 5-thymyl radical increase.

This indicates that, upon warming, some of the 6-thymyl radicals are converted into 5-thymyl radicals.

It was reported earlier[14] that the 6-thymyl radical converts into the 5-thymyl radical in the temperature range 90°C to 130°C. The activation energy for this process was found to be 23.1 kcal/mole, with a frequency factor of 1.2×10^{12}/min. An extrapolation of these values to the temperature range 77°K to 295°K shows that the conversion should be entirely negligible at these temperatures. Thus, it appears that an additional conversion process, with different kinetics, occurs below 295°K. The mechanism(s) for free radical conversion is not known in either case.

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Table I. Principal values of the spectroscopic splitting tensor (g) and the α-hydrogen hyperfine tensor (A^{α}) for the 6-thymyl radical in irradiated dihydrothymine. Direction cosines relating the corresponding principal axes to the crystallographic abc coordinate system are given for one molecule in the unit cell.

	Principal value	Direction cosines		
		a	b	c
g_u	2.0039	0.40	0.76	0.51
g_v	2.0031	-0.47	-0.31	0.82
g_w	2.0022	0.79	-0.57	0.23
A^{α}_x	7.8 gauss	-0.20	0.37	0.90
A^{α}_y	26.9 gauss	-0.42	-0.87	0.26
A^{α}_z	17.5 gauss	0.89	-0.36	0.33
A^{β}	44.0 gauss	Isotropic		

Figure Captions

Fig. 1. Second-derivative ESR spectrum of an irradiated single crystal of dihydrothymine, oriented with the external magnetic field along the crystallographic b axis. Vertical arrows indicate the outer components of the hyperfine spectrum of the 5-thymyl radical. Bars beneath the spectrum represent the quartet hyperfine pattern of the 6-thymyl radical, with coupling constants calculated from spin resonance parameters given in Table I. The observation was made at room temperature, at a microwave frequency of 16 GHz.

Fig. 2. ESR spectrum of irradiated dihydrothymine, as described in Fig. 1, but with the magnetic field along the a axis. At this orientation, the absorption of the 5-thymyl radical is very weak.

Fig. 3. Comparison between the calculated (solid lines) and observed (circles) values for the spin resonance parameters of the 6-thymyl radical in irradiated dihydrothymine. The calculations are based on tensor components given in Table I. The magnetic field is in the crystallographic ab plane.

Fig. 4. Calculated and observed values as described in Fig. 3, but with the magnetic field in the ca plane.

Fig. 5. Second-derivative ESR spectra of a single crystal of dihydrothymine, irradiated and observed at 77°K. The magnetic field is (a) along the b axis and (b) along the a axis. The observation frequency was 9 GHz.

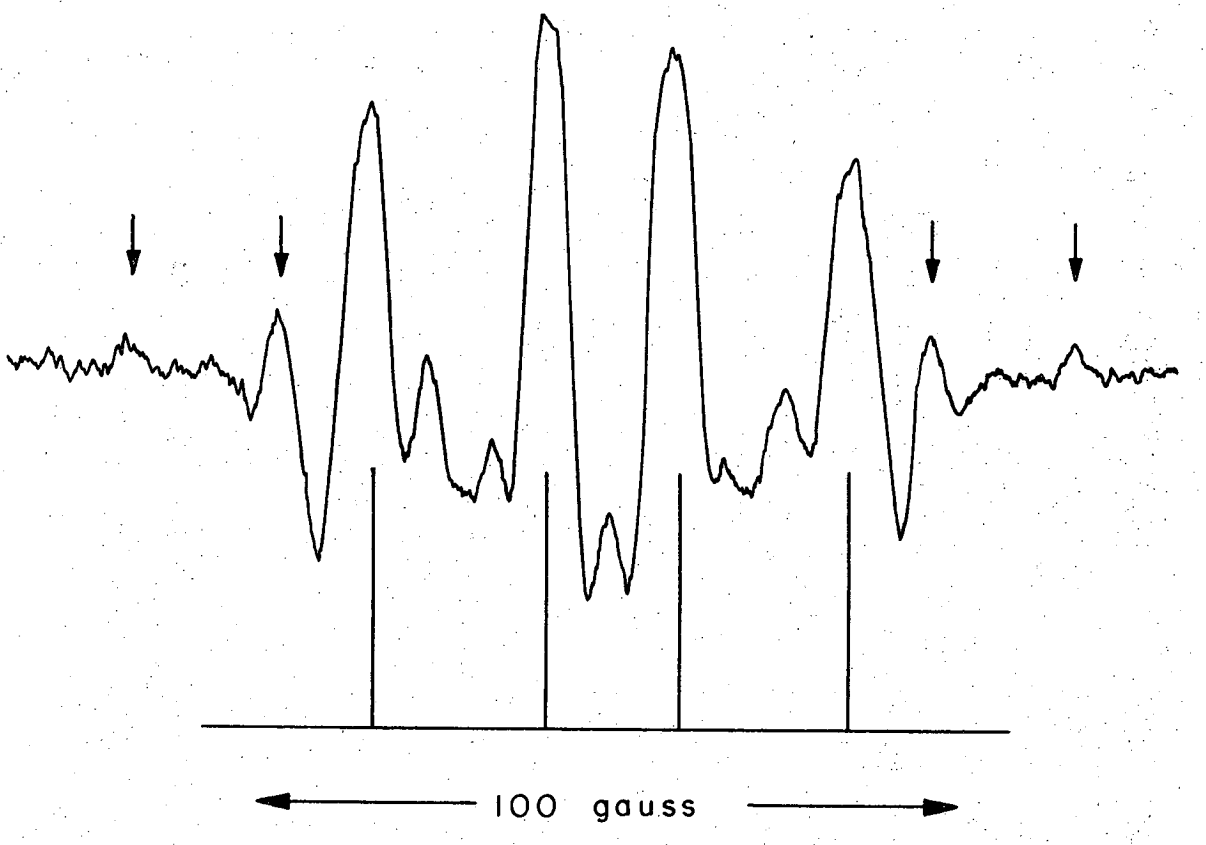

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Fig. 1

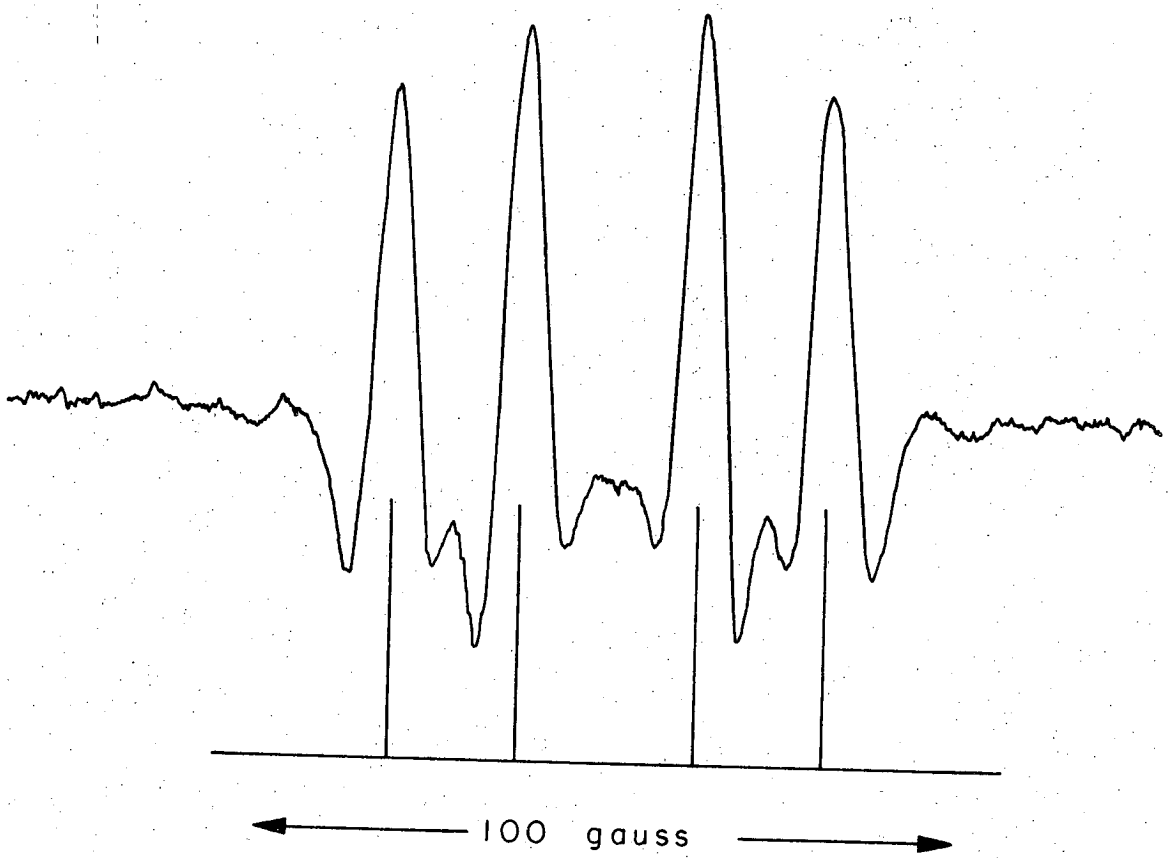

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Fig. 2

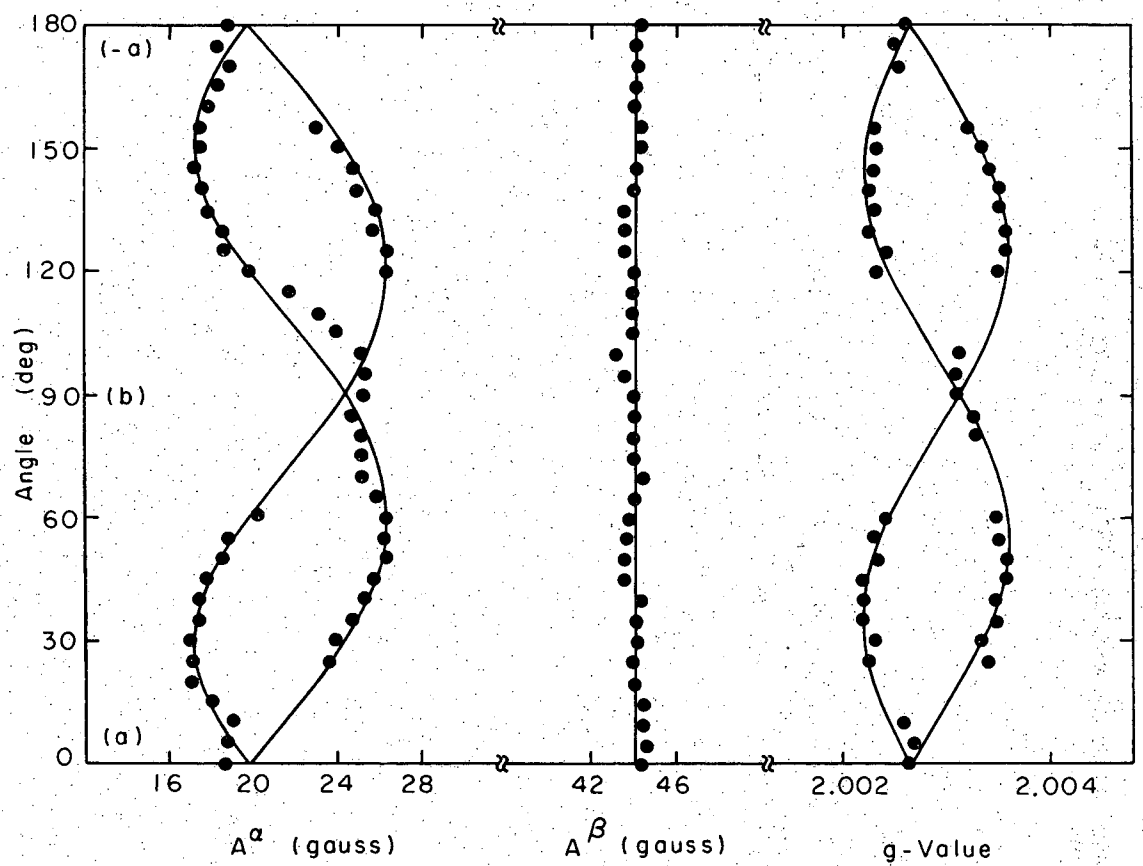

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Fig. 3

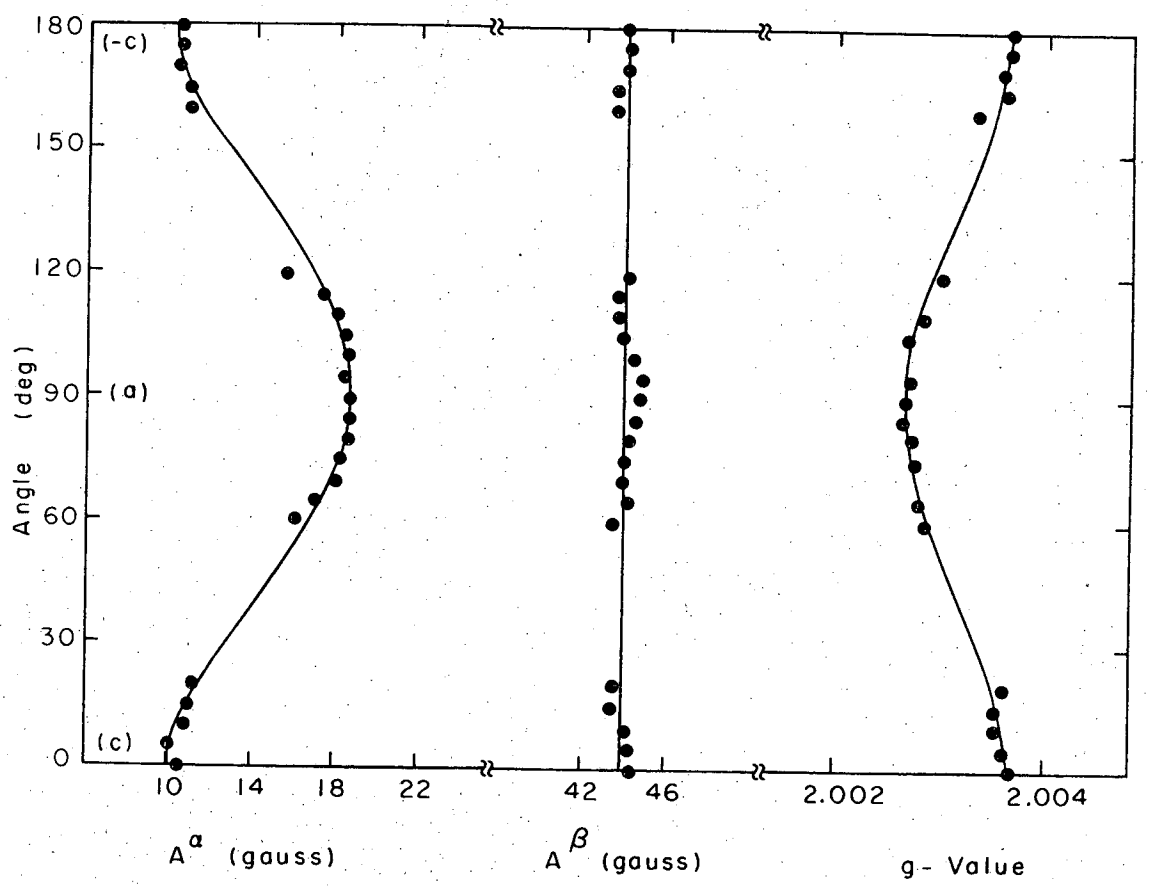

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Fig. 4

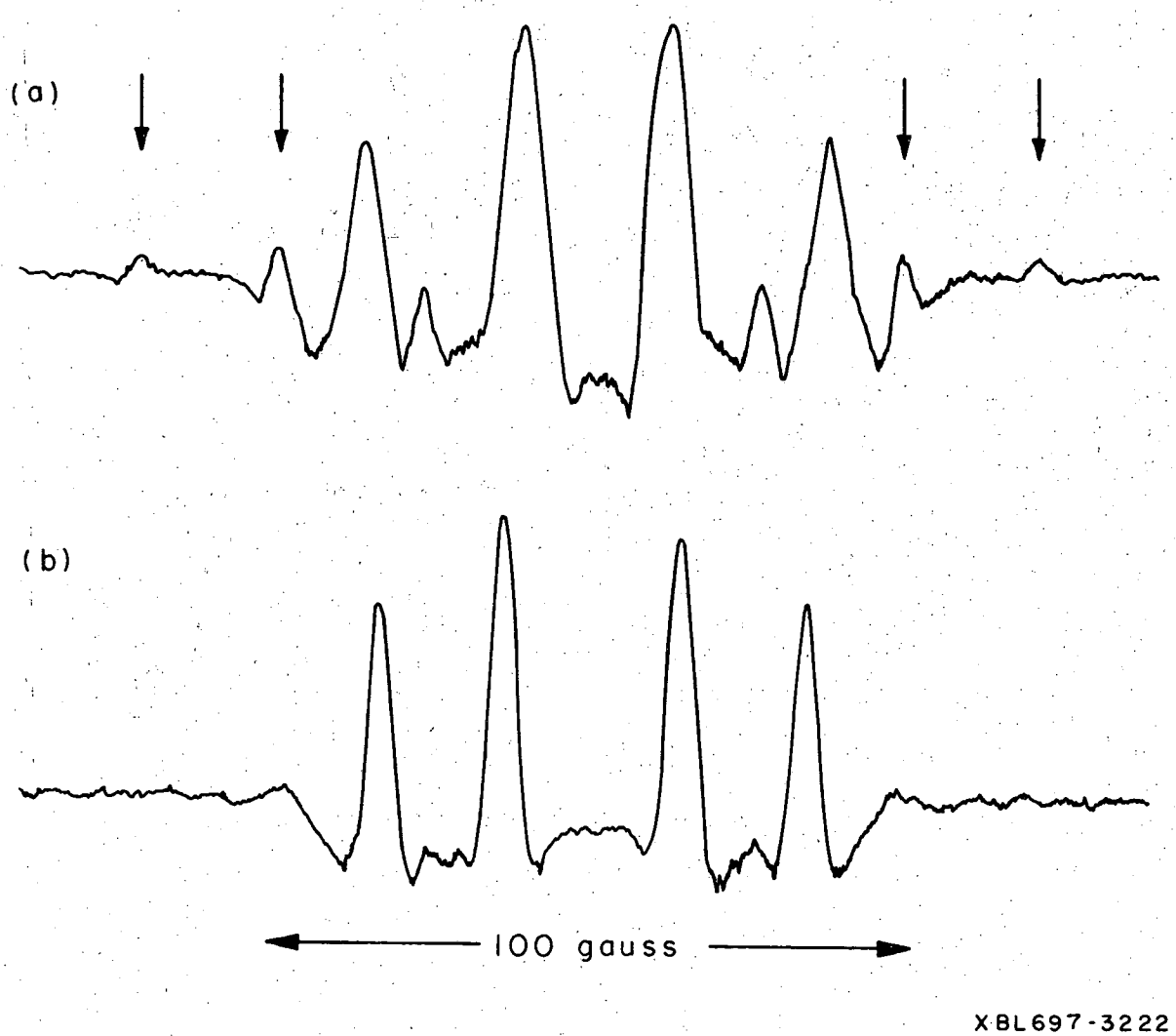

Fig. 5

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