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ELECTRON SPIN RESONANCE STUDIES OF FREE RADICALS
FORMED FROM OROTIC ACID¹

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Abstract

The paramagnetic species produced in orotic acid by irradiation of single crystals and polycrystalline material as well as by reaction with hydroxyl free radicals in an aqueous system have been studied by electron spin resonance (ESR) spectroscopy. Three species were observed in the spectra of the crystals, two of them free radicals associated with the orotic acid molecule and the third, cupric ions resulting from radiation effects on crystal impurities. The spectra of the latter were not investigated in full detail. One of the free radicals is formed by hydrogen addition to the 5-position of the pyrimidine ring, leaving an unpaired electron in a $2p_z$ -orbital of carbon atom $C_{(6)}$. The couplings of the two β -hydrogen atoms at the 5-position are 22.8 and 34.2 Gauss respectively. The principal values of the g-tensor are 2.0023, 2.0066, and 2.0031. The second radical results from a hydrogen added to an oxygen of the carboxyl group, giving rise to interaction of the radical electron on carbon $C_{(7)}$ with two hydroxyl protons. The hyperfine couplings of the latter are almost axially symmetrical with one $A_{||} \simeq 12$, $A_{\perp} \simeq 0$ Gauss, and the other $A_{||} \simeq 6$, $A_{\perp} \simeq 0$ Gauss. The g-tensor of this radical has principal values of 2.0022, 2.0090, and 2.0047. The spectra obtained from irradiated polycrystalline orotic acid can be explained on the basis of the same two radicals. Hydroxyl free radicals produced by the $Ti(III) - H_2O_2$ reaction add to the 5-position of orotic acid in acidic aqueous solution. The unpaired electron on carbon $C_{(6)}$ interacts with the β -proton on $C_{(5)}$ to produce a doublet of 11.8 Gauss spacing. Each doublet line is further split into three main lines with component spacing of 2.3 Gauss by interaction with the ^{14}N nucleus in 1-position.

Introduction

When nucleic acid constituents in the solid state are exposed to ionizing radiation at room temperature, a major pathway of radiation damage has been shown by electron spin resonance (ESR) spectroscopy to be the addition of a hydrogen atom to the unsaturated bonds of the ring of the base molecule.³⁻⁹ Formation of this kind of radical is the predominant type of base damage in purines. However, other radicals are found in significant amounts in pyrimidines. Recent single crystal studies have revealed, that in a number of pyrimidine derivatives a radical is formed which involves hydrogen atom addition to one of the keto oxygens.^{5,7,10} Production of such radicals has interesting radiobiological implications since it would cause destruction of the hydrogen bonding scheme in double-stranded DNA. A third type of pyrimidine radical is generated by reactions involving substituents at the 5,6-double bond. Thus, in thymine a hydrogen atom is removed from the 5-methyl group.⁶

To obtain more information about the various mechanisms of radiation damage in pyrimidine derivatives we extended our investigations to orotic acid, a compound which is derived from uracil by replacement of the 6-hydrogen by a carboxyl group. ESR spectra of irradiated single crystals were used to identify the radicals formed in the solid state. The studies were complemented by investigation of irradiated polycrystalline material and of the radicals formed by reaction of hydroxyl free radicals with the compound in an aqueous system.

Experimental Section

Single crystals of orotic acid monohydrate (Calbiochem A grade) were grown from aqueous solutions as plates, weighing about 10 mg, by repeatedly

seeding small crystals in fresh mother liquor. The average crystal dimensions obtained were about 5 x 2 x 1 mm. To our knowledge no data have appeared on the crystal structure and molecular parameters of this compound. Preliminary Weissenberg diagrams reveal that the crystals are triclinic with space group $P\bar{1}$ and two molecules per unit cell assuming a density of 1.5. The two molecules are related by a center of symmetry. In analogy to uracil and thymine it can be assumed that the molecule is in the diketo form. Fig. 1 shows the orotic acid molecule and the numbering system used in this paper, as well as the external form of the crystals and the orthogonol abc-axis system used for ESR measurements.

It should be noted that the relatively large crystals used in this investigation exhibited a slightly yellow color although the small seeding crystals appeared colorless.

The crystals were exposed to radiation from a ^{60}Co - γ -source at doses between 10 and 60 MR. ESR spectra were recorded as first derivatives of the absorption using a Varian 4502 spectrometer operating at about 9 GHz (X-band) microwave frequency. The three lines of Fremy's salt ($g = 2.0055$, $\Delta H = 13$ Gauss) were employed as a standard for calibrating g -factors and hyperfine splittings. The spectral data were evaluated by the procedure of Schonland¹¹ and Lund and Vänngård.¹² Calculations were made on an IBM 360 Computer.

Hydroxyl free radicals were generated in aqueous solution with the $\text{Ti(III)} - \text{H}_2\text{O}_2$ system following mainly the standard procedure given by Dixon and Norman.¹³ The solutions were 0.004 M with respect to orotic acid. To avoid competition of H_2O_2 for OH radicals, concentrations of 0.015 M H_2O_2 and 0.005 M Ti(III) were used. OH radicals react with

chloride ions in acid solution to give Cl_2^- ion radicals^{14,15} which have an intense ESR signal interfering with the lines from solute radicals. Thus we used $\text{Ti}_2(\text{SO}_4)_3$ (Lamotte Chem. Prod) as Ti(III) source instead of TiCl_3 which is usually employed. All solutions were thoroughly bubbled with nitrogen gas. The pH was adjusted with conc. H_2SO_4 and was measured with a Beckman pH-meter before and after mixing of the two reactants.

Results and Discussion

Three different paramagnetic species, labeled A, B, and C are observed in the irradiated orotic acid single crystals as is shown in the ESR spectrum of Fig. 2. Two of the species (A and B) are stable for at least several months and display a superimposed hyperfine spectrum near $g = 2$ which is typical for organic free radicals. The spectrum of the third, C, which is represented by the first four lines at low field in this orientation, decays slowly at room temperature and appears at much higher g -factors.

Radical Species A

The $g = 2$ section of room temperature spectra of orotic acid crystals irradiated with about 40 MR is shown for two orientations of the crystal in Fig. 3. The magnetic field is in the ab -plane of the crystal, the angle between the two orientations being 120° . In the left spectrum, a group of four lines, labeled B, is seen to be superimposed on a spectrum with broader lines, the outer components of which are marked by arrows. The number of lines of the latter group, which is referred to as A in the following cannot be inferred from the left spectrum of Fig. 3. In the spectrum to the right, the four lines of B have collapsed to a singlet and a third line of A is seen to appear, marked by a double arrow. Since this line is not placed in the center of the two outer lines of A, symmetry sug-

gests that the number of lines in Group A is four. This is indicated by the bars given under the spectrum to the right in Fig. 3. The fourth line appears as a shoulder on the singlet.

Variation of the incident microwave power or the temperature of ESR measurements of crystals irradiated at room temperature does not separate the lines of A and B. Likewise, heat-annealing at 510°K for several minutes (which did not visibly damage the crystals) and storage of the annealed crystals for a week decreased both line groups equally. However, irradiation of the crystals at 77°K and measurements at 170°K or 300°K results in spectra in which the $g = 2$ section consists dominantly of the lines of species A, as is shown in the spectrum of Fig. 4 (cf. the legend for details of experimental conditions). Indeed, four lines of about equal intensity are seen to represent group A. On rotation of the crystal in the ab-plane the splitting of these lines is nearly isotropic. This is in accord with the isotropic total spacing of the lines of group A as measured by the distance of its outermost lines (cf. arrowed lines in spectrum of Fig. 3) in the spectra of the crystals which were irradiated at room temperature. These findings indicate that the unpaired electron of the radical giving rise to group A interacts with two protons in β -position with couplings of $a_{H\beta}(I) = 22.8$ Gauss and $a_{H\beta}(II) = 34.2$ Gauss.

The width of the lines of group A is subject to marked changes with crystal orientation. Fig. 5 shows this variation for the two outer lines of A on rotation of the crystal in the ab-plane. Because of the angular π -symmetry of the line width variation in this plane, unresolved site splitting of magnetically unequivalent molecular sites in the crystal cannot account for this feature. Instead, poorly resolved anisotropic

hyperfine splitting resulting from interaction of the unpaired electron with other nuclei is assumed to be present.

Fig. 6 shows the variations of the g-factors for group A in the three crystal planes, measured from the position of the center of the two outer lines of this group. The g-tensor is characteristic of a radical with its unpaired electron localized mainly in a carbon $2p_z$ orbital.

All features of group A can be accounted for by assuming that the corresponding radical is formed by addition of a hydrogen to the 5-position of the 5,6-double bond of the orotic acid molecule, leaving an unpaired electron in the $2p_z$ -orbital of ring carbon $C_{(6)}$ (radical II).

The hyperfine spectrum arises from the interaction of the electron with the two methylene protons attached to carbon $C_{(5)}$. The additional unresolved splitting may in part result from the interaction with the ^{14}N nucleus in the 1-position.

The two β -protons on $C_{(5)}$ do not couple equally. Assuming sp_3 -bonding to $C_{(5)}$ the dihedral angle θ of the $C_{(5)}-H$ direction with respect to the plane perpendicular to the π -orbital of the unpaired electron must be different for each of the two protons. The angles are related by

$$\theta_2 = 60^\circ - \theta_1.$$

The couplings of β -hydrogens are given by the relation of Heller and McConnell¹⁶

$$a_{H\beta} = \zeta_{C\alpha} \cdot A_o \cdot \cos^2 \theta_1$$

with the constant $A_o \simeq 58.5$ Gauss and $\zeta_{C\alpha}$ the spin density on the α -carbon. Insertion of the numerical values of the two couplings yields

$$\theta_1 = 41^\circ \quad \theta_2 = 19^\circ \quad \zeta_{C\alpha} = 0.67$$

The comparatively small value of $\zeta_{C\alpha}$ can be explained satisfactorily by resonance structure (III) of radical (II) in which some unpaired spin density is localized on the carboxyl oxygen.

Since the hyperfine couplings of radical (II) have proven isotropic in the single crystal, irradiated polycrystalline material of orotic acid should yield roughly the same spectrum for this group as the crystal spectra. Fig. 7 shows the spectrum obtained from polycrystalline orotic acid exposed to 6 MR at room temperature. It consists of four lines with spacings approximately equal to those of group A lines in the crystal (cf. Fig. 4). Two major differences are apparent. The center lines are unequal in intensity and are themselves larger than the two outermost lines. The latter effect is explained by g-factor averaging resulting from random orientations of the radicals in the polycrystalline material while the former is due to the presence of radical species B as will be discussed in the next section. Therefore, both the spectra of the single crystals and of the polycrystalline material are compatible with the proposed structure (II) for the lines of group A.

A spectrum of four hyperfine lines with about the same component splittings as in Fig. 7 (18 and 34 Gauss) has been reported for orotic acid exposed to thermal hydrogen atoms¹⁷ and attributed to a radical

resulting from hydrogen addition to the 6-position of the pyrimidine ring.

The similarity of the hyperfine couplings to those reported here, suggest that the hydrogen atoms were at the 5-position in that case also.

The ESR spectrum obtained from the reaction of hydroxyl free radicals with orotic acid in acid aqueous solution is shown in Fig. 8a. Variation of the pH from 1.6 to 2.5 produced little spectral change. Since these values are close to the pK value of the carboxyl group of orotic acid (2.4), either i) the ionization of the carboxyl group does not affect the distribution of the unpaired electron, or ii) the pK value of the radical resulting from OH attack differs greatly from that of the parent compound. The spectrum consists of two main hyperfine lines with a spacing of 11.8 Gauss centered at a g-factor of 2.0035. Fig. 8b gives the trace of one of the doublet lines recorded at a slower scan speed under conditions of maximum resolution and using a computer of average transients. Three lines of about equal intensity with component spacing of 2.3 Gauss are seen to constitute each of the doublet lines. The spectrum is therefore assigned to the same type of radical as structure (II) with the difference that one of the hydrogen atoms at the 5-position is, of course, replaced by a hydroxyl group. The triplet splitting then stems from interaction of the unpaired electron on carbon atom C₍₆₎ with the nuclear spin of the nitrogen in the 1-position, whereas the main doublet hyperfine spectrum is due to coupling of the β -proton attached to carbon atom C₍₅₎. The rather small value of this coupling (11.8 Gauss) can be accounted for by assuming that there is a low spin density on C₍₆₎ because of contributions of resonance structures of the type (III), and that the planar ring structure of the orotic acid molecule is distorted upon attachment of the hydroxyl group. The direction of this

distortion is such, that the dihedral angle of the $C_{(5)}-H$ bond with the π -orbital axis on $C_{(6)}$ is closer to 90° than it is after addition of a hydrogen atom. The latter effect has also been observed for reactions of hydroxyl free radicals with other pyrimidine bases in aqueous solution.¹⁸

The two satellite lines of minor intensity which appear to the left and right of the center line of the triplet (Fig. 8b) do not fit into the radical scheme outlined above. They cannot be due to addition of hydroxyl free radicals to another ionic form of orotic acid since their intensity does not vary over the pH-range investigated. These lines may result from an additional radical which, however, must have the same main doublet splitting and g-factor as that described above. A radical of a different chemical structure meeting these requirements is most unlikely. Therefore, we propose that a geometrical isomer of the same OH adduct radical of type (II) is present. The source of this isomerism may be the non-planarity of the radical.

Radical species B.

In contrast to the isotropic behaviour of the lines of radical (II) the hyperfine spectrum of species B in the single crystals varies markedly with orientation (Fig. 3). The number of lines belonging to this group was found to be one, two, three or four. The lines of the doublet and the quartet patterns have about equal intensities; those of the triplet have an intensity ratio 1:2:1.

Fig. 9 shows the variations of the lines with orientation. When they approach, their position becomes uncertain because of the linewidths. This uncertainty is minimized by a numerical least squares fit of the experimental values in deriving the hyperfine splittings from these data. The lines are symmetric around one center of gravity for all orientations

in the three planes. Thus, one radical accounts for all lines. This is in agreement with the results of varying the microwave power and the temperature of measurement, as well as with heat annealing experiments. Furthermore, the variations of all lines in each plane exhibit angular π -symmetry. Therefore, the radical must occupy only one site in the crystal. Examination of the lines in all crystal planes reveals that they are due to the interaction of the unpaired electron with two protons. Fig. 10 gives the variations of the splittings of these protons H_1 and H_2 (cf. radical IV) in the three crystal planes. The couplings are approximately axially symmetric with values between about 0 and 6, and 0 and 13 Gauss respectively. These values are characteristic for protons adjacent to an oxygen atom in a $\text{>}\dot{\text{C}}\text{-OH}$ radical fragment. The only position in the orotic acid molecule which can account for these features is the carboxyl-group. Therefore, the radical under consideration is proposed to be of the form (IV).

The variations of the g-factors of radical (IV) in the three crystal planes are shown in Figure 11. Comparison with the data of Figure 6 show that the g-factors of radical (IV) are slightly higher than those of the hydrogen addition radical. This effect is expected for $\text{>}\dot{\text{C}}\text{-OH}$ vs. $\text{>}\dot{\text{C}}\text{-C}\equiv\text{H}_\beta$ radical fragments.¹⁹

For axial symmetry of both hydroxyl proton couplings in radical (IV) the isotropic components $a(I)$ and $a(II)$, and the anisotropic components $b(I)$ and $b(II)$ of the couplings can be obtained from the equation

$$A_{\parallel} = a + 2b$$

$$A_{\perp} = a - b$$

with $A_{\parallel}(I) = 12.1$ Gauss, $A_{\perp}(I) \cong 0$ Gauss and $A_{\parallel}(II) = 5.96$ Gauss and $A_{\perp}(II) \cong 0$ Gauss. (Both $A_{\perp}$ values of 0 Gauss are estimates since they cannot be inferred positively from the spectra because of the width of the lines). The components obtained by the above equation are $a(I) = b(I) = 4.03$ Gauss and $a(II) = b(II) = 1.98$ Gauss.

The anisotropic component $b(I)$ can be used for an estimate of the spin-density, $\zeta_{C_{\alpha}}$, assuming that this dipole coupling arises only from unpaired electron density on carbon $C_{(7)}$. Using the equation²⁰

$$b(I) = 28.2 \cdot \zeta_{C_{\alpha}} / r^3$$

and taking r as $1.74 \text{ \AA}$, $\zeta_{C_{(7)}}$ is found to be 0.74. The dihedral angles θ_I and θ_{II} for the two OH bonds with respect to the π -electron orbital can be found by inserting the isotropic hydroxyl proton couplings $a(I)$ and $a(II)$ into the relation¹⁹

$$a = (B_0 + B_1 \cos^2 \theta) \cdot \zeta_C$$

With the constants $B_0 = -2.5$ Gauss and $B_1 = 40$ Gauss we obtain

$$\theta_I \simeq 63^\circ \text{ and } \theta_{II} \simeq 70^\circ$$

Table II gives the data for the g -tensor and the hyperfine couplings of radical (IV) together with the direction cosines.

It remains to discuss the difference in the proton couplings of the two hydroxyl groups adjacent to carbon $C_{(7)}$ in radical (IV). The differences in the isotropic components of the couplings must result from differing

dihedral angles which the OH bonds make with the Π -orbital on C_α . The anisotropic components of the hydroxyl proton coupling is proportional to the inverse cube of the distance r between the proton and the radical electron on C_α . In the undamaged orotic acid molecule, both oxygens of the carboxyl group take part in hydrogen bonding to neighbor molecules. It can be assumed that, in the corresponding radical (IV), one hydrogen bond is still intact whereas the other has been broken upon radical formation. Thus, the distances r_1 and r_2 would be different for the two hydroxyl protons with r being longer for the proton which takes part in hydrogen bonding.

It is not clear, whether the mechanism of formation of this radical is associated with addition of atomic hydrogen to the oxygen. Possibly, it is due to a protonation of a negatively charged radical. The proton may well stem from the hydrogen bond by which the oxygen is bound to a neighboring molecule. This assumption draws support from the fact, that exposure of orotic acid in the polycrystalline state to thermal hydrogen atoms does not result in the formation of radical (IV). Instead, only the addition reaction to the 5,6-double bond takes place.¹⁷

No quantitative investigations of the concentrations of the different species in the spectra have been carried out, but the yield of radical (IV) appears to be much higher than that of (II) when the crystals are irradiated at room temperature. Low temperature irradiation, however, reduces its contribution strongly.

In the spectrum of the irradiated polycrystalline sample of orotic acid (Figure 7), radical (IV) is present, too. The hydroxyl proton hyperfine splittings collapse to a singlet under these conditions, which, because

of the higher g-factor overlaps mainly with the second left line of radical (II) causing the intensity unsymmetry of the spectrum which was mentioned in the previous section.

Species C

A group of four hyperfine lines, labeled C, is present in the spectra of irradiated orotic acid single crystals at both 77°K and room temperature (cf. Fig. 2 for one 300°K spectrum). This group appears at much higher g-factors than the lines of groups A and B, reflecting a strong spin-orbit interaction which leads to the assumption that the spectrum must be due to a paramagnetic ion with nuclear spin 3/2. Since neither the unirradiated crystals, nor the unirradiated or irradiated polycrystalline orotic acid display this hyperfine spectrum, it must result from an impurity in the single crystals whose paramagnetism becomes apparent as a result of the irradiation. Emission spectra of the single crystals revealed the presence of about 70 ppm copper as the largest impurities.

Our investigation of the lines of group C in the ab-plane of the single crystals were sufficient to make certain that the four line spectrum arises from single Cu^{2+} ions. The g-factor varies between 2.065 and 2.166 and the hyperfine spacing of the quartet components between 44 Gauss and 108 Gauss in this plane. Fig. 12 shows the four lines in an orientation with maximum hyperfine splitting. The outer quartet lines are seen to be split into doublets due to the difference of the magnetic moments of the isotopes ^{63}Cu and ^{65}Cu . No further analysis of the Cu^{2+} spectrum is attempted here since the $S = 1/2$, $I = 3/2$ system of cupric ions has been investigated recently in an irradiated single crystal of cupric acetate.²¹

We assume that the copper is an impurity in the mother liquor. Since at neutral pH orotic acid is ionized, some molecules may crystallize as the non-magnetic cuprous orotate. Radiation can then convert cuprous to cupric ions, which would give rise to the observed spectra. Possibly, the presence of cuprous ions causes the slight yellow color of the unirradiated orotic acid single crystals which was mentioned in the experimental section.

Summary

Of the three paramagnetic species observed in irradiated single crystals of orotic acid, two have been shown to be free radicals involving the orotic acid molecule whereas the third is the result of a copper impurity present during crystallization. One radical, structure (II), is formed by hydrogen addition to the carbon atom C₍₅₎. The source of the attaching hydrogen is not known since no hydrogen abstraction radical was observed. This is not surprising, however, since in nearly all unsaturated pyrimidine bases hydrogen addition at the 5,6-double bond takes place without revealing the source of the hydrogen atoms. In aqueous solution hydroxyl free radicals add to the 5,6-double bond at the 5-position to give the OH analog of structure (II). The other radical observed in single crystals of orotic acid, structure (IV), involves addition of a hydrogen to the oxygen of the carboxyl group. Again, the source of the hydrogen is not known. The spectra obtained from polycrystalline orotic acid can be explained on the basis of radicals (II) and (IV).

While this report was being prepared, an ESR study of irradiated single crystals of orotic acid was published by Horan and Snipes.²² On the basis of spectra differing from those reported here, these authors conclude that only one radical is present, and that it results from abstraction of a hydrogen atom from the nitrogen in the 1-position. Surprisingly, no hydrogen addition to the 5,6-double bond is reported. We assume, that the differing ESR spectra result from differences in the crystals investigated. Such differences are obvious, since these authors report the occurrence of magnetically inequivalent molecules which are not observed in the triclinic crystals analyzed here. The crystals grown by Horan and Snipes from a mixture of ethanol and 0.8 M NH_4OH most probably are the diammonium salt of orotic acid.

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Table I. Characteristics of ESR spectra of radical (II) in orotic acid under the various experimental conditions

a) Single crystals				
	Principal Values	Direction cosines		
		a	b	c
g	2.0023	0.85	0.51	-0.10
	2.0066	-0.47	0.69	-0.59
	2.0031	-0.20	0.51	0.84
$a_{H\beta}$ (I)	22.8 Gauss	isotropic		
$a_{H\beta}$ (II)	34.5 Gauss	isotropic		
b) $OH\cdot$ + Orotic acid in aqueous solution (pH = 1.6 - 2.5)				
g	2.0035			
$a_{H\beta}$	11.8 Gauss			
$a_{N\beta}$	2.3 Gauss			

Table II. Principal values of g-tensor and hyperfine splittings (in Gauss) of radical (IV) in single crystals of orotic acid.

	Principal Values	Isotropic Component	Direction Cosines		
			a	b	c
g	2.0022		0.92	0.38	-0.01
	2.0090		-0.36	0.76	-0.54
	2.0047		-0.15	0.53	0.84
$A_{OH}^{(II)}$	$A_{ } = 5.96$	1.98	0.14	0.53	0.84
	$A_{\perp} \simeq 0$		-0.49	0.78	-0.40
$A_{OH}^{(I)}$	$A_{ } = 12.1$	4.03	0.81	0.43	-0.41
	$A_{\perp} \simeq 0$		-0.59	0.66	-0.46

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- (2) On leave from: Lehrstuhl für Physik, Fachbereich Biologie
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Fig. 1: Representation of orotic acid molecule and orotic acid monohydrate single crystal showing numbering system and orthogonal ESR reference axes a, b, and c respectively.

Figure 1

Fig. 2: First derivative ESR spectrum of orotic acid crystal exposed to about 40 MR at ambient temperature showing three distinct line groups A, B, and C. Spectra are taken at X-band frequency at 300°K. Magnetic field H is in ab-plane.

Figure 2

Fig. 3: Free radical section of ESR spectra of orotic acid crystals for two different orientations showing species B and A as indicated by the bars beneath the left and right spectrum. Experimental conditions otherwise as in Fig. 2.

Figure 3 (left part)

Figure 3 (right part)

Fig. 4: First derivative ESR spectrum of orotic acid crystal exposed to 15 MR at 77°K, stored at 300°K for several days, and re-irradiated at 77°K with 20 MR. Temperature of measurement is 300°K. Magnitudes of coupling $a_{H\beta}$ (I) and $a_{H\beta}$ (II) refer to radical structure (II) for species A.

Figure 4

Fig. 5: Variation of width of outer lines of group A with magnetic field H in ab -plane of orotic acid crystals.

Figure 5

—Fig. 6: Variation of g-factors of group A in three planes of orotic acid single crystals. The open circles are the experimental points, the solid lines the theoretical curves based on the data given in Table I.

Figure 6

Fig. 7: First derivative ESR spectrum of polycrystalline orotic acid exposed to 6 MR at 300°K. Spectrum taken at X-band frequency at room temperature.

Figure 7

- Fig. 8:
- a) First derivative ESR spectrum produced by reaction of OH· free radicals with orotic acid in aqueous solution at pH = 2.4. Arrow indicates position of center line of Fremy's salt ($g = 2.0055$).
 - b) Trace of high field line of main doublet of spectrum in a) at slower scan rate and with optimized resolution.

Figure 8a

Figure 8b

—Fig. 9: —Variation of all hyperfine lines of group B in three planes of orotic acid single crystals. Open circles represent the positions of the lines with regard to the magnetic field given as the abscissa of the curves.

Figure 9

—Fig. 10: Variation of hydroxyl proton splittings of proposed radical structure (IV) (of group B) in three planes of orotic acid single crystals. The open circles and the crosses are the experimental values of proton couplings H_1 and H_2 respectively, the solid lines represent the calculated curves.

Figure 10

Fig. 11: Variation of g-factor of radical structure (IV) (of group B)
in three crystal planes of orotic acid single crystals.

Figure 11

Fig. 12: First derivative ESR spectrum of group C in single crystals of orotic acid showing Cu^{2+} hyperfine lines and isotopic splitting of ^{63}Cu and ^{65}Cu nuclei.

Figure 12

(I)

(II)

(III)

(IV)