# Temperature-Dependent ESR Studies of Radical Pairs in Single Crystals of Barbituric Acid

Robert Schupfner and Adolf Müller

Institut für Biophysik und Physikalische Biochemie, Universität Regensburg,
Postfach 397, D-8400 Regensburg, Bundesrepublik Deutschland

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After irradiation of single crystals of barbituric acid with X-rays at 77 K different types of radical pairs are found, which are composed of only one type of monoradical. The properties of radical pairs of the unpaired electrons are studied using frequency-variable ESR methods at various temperatures. In addition to the radical pairs AD and BC two further pairs A′D′ and XY were identified. Measurements of the fine structure parameter D showed a linear temperature dependence in some regions between 77 K and 290 K. At 240 K the radical pairs AD and A′D′ changed reversibly into A*D* and this pair remained stable up to 290 K.

A level anticrossing effect was observed with the pair AD. The exchange energy *J* between the singlet state and the triplet state was determined as $-(15.1 \pm 0.6)$ GHz at 77 K and its temperature coefficient as $-(3.8 \pm 0.8) \times 10^{-3} \mathrm{K}^{-1}$.

## Introduction

When single crystals of barbituric acid are exposed to X-irradiation radicals are formed. The main process of radical production at 77 K can be described by the net reaction shown in Fig. 1. Most of these are isolated monoradicals but some are located in close proximity forming paired radicals [1, 2]. In an external magnetic field radical pairs are represented by triplet states as shown in Fig. 2 for barbituric acid. Electron spin resonance (ESR) spectroscopy of these crystals yields absorption lines due to pairs ($\Delta M_s \pm 1$) which are situated symmetrically on both sides of the magnetic field strength associated with the monoradical absorption. The observed fine structure splitting $\Delta$ is strongly anisotropic due to the dipolar interaction of the paired electrons. From the directional variation of the observed splitting $\Delta$ the fine struc-

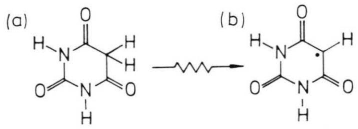

Fig. 1. Formation of the monoradical at position C5 by irradiation with X-rays [1].

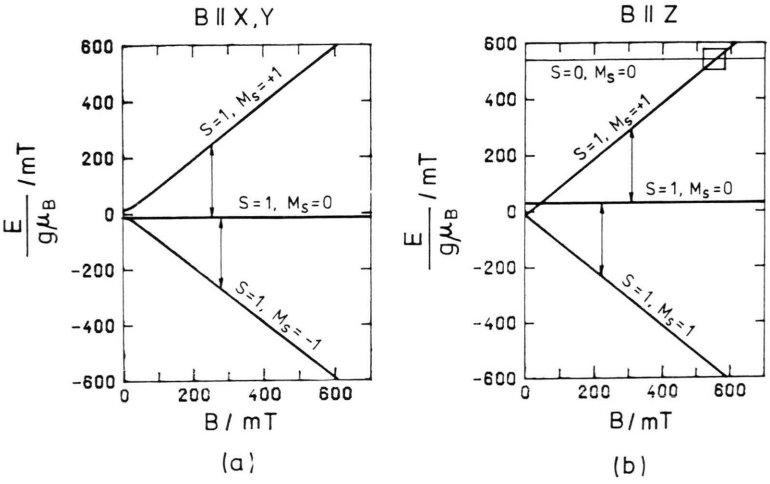

Fig. 2. Energy levels and ESR transitions of radical pairs in single crystals of barbituric acid. Magnetic field B parallel to the *x*- or *y*-axis of splitting tensor (a), and parallel to the *z*-axis (b).

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Reprint requests to Prof. Dr. A. Müller-Broich.

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ture constants $D$ and $E$ can be derived. The splitting $\Delta$ between the centre of hyperfine lines at low and high field strengths can be described by the Eqn. [3]:

$$g\mu_B\Delta = D(3\cos^2\Theta - 1) + 3E\sin^2\Theta\cos^2\Theta. \quad (1\alpha)$$

$\Theta$ and $\Phi$ denote the azimuthal and equatorial angles between the vector linking the monoradicals of a pair and the direction of the magnetic field.

If both vectors are parallel (principal axis $z$) one obtains

$$g\mu_B\Delta = -2D. \quad (1\text{b})$$

In addition to orientation of the crystal in the magnetic field the dipolar splitting also depends on the mutual distance $R$ of paired electrons as $R^{-3}$ [4–6]. Small changes of this parameter due to thermal deformations result in corresponding changes of the fine structure splitting. The thermal variation of the fine structure constant $D$ was described by Flossmann *et al.* [4] as

$$\delta D = D_0\gamma\delta T \quad (2)$$

where $D_0$ is the fine structure constant at a fixed temperature (*e.g.* 77 K) while $\gamma$ is the temperature coefficient.

In addition to the dipolar interaction the energy of the system is modified by exchange interaction between overlapping orbitals of the unpaired electrons [3]. The exchange energy $J$ is even more sensitive against changes of distance between the interacting electrons than the dipolar energy. Such changes are caused by thermal deformations of the crystal [4, 5, 7]. In a limited temperature range (94 K to 111 K) a linear relation between $J$ and the temperature change $\delta T$ was reported by Flossmann *et al.* [4]

$$J(T) = J_0(1 + \beta\delta T). \quad (3)$$

$J_0 = J(77\text{ K})$, $\beta$ is the temperature coefficient of the exchange energy.

The exchange energy $J$ of radical pairs in single crystals was determined for 1-methyl uracil (1-MU) by Flossmann *et al.* [4, 8] and for potassium hydrogen malonate (KHM) by Knopp [3].

In the present work the exchange energy and its variation with temperature in another single crystal, that of barbituric acid, is measured. Here, the pyrimidine rings are stacked in parallel layers as in 1-MU [9] whereas the hyperfine structure results from a pair of $\alpha$-protons as in KHM. Fine structure lines of pairs are further split by nuclear hyperfine interaction. In the simplest case as in barbituric acid and KHM this is caused by two interacting $\alpha$-protons, one on each radical [2].

In addition to the thermal variation of the exchange energy, the corresponding effect on the fine structure constant $D$ is determined.

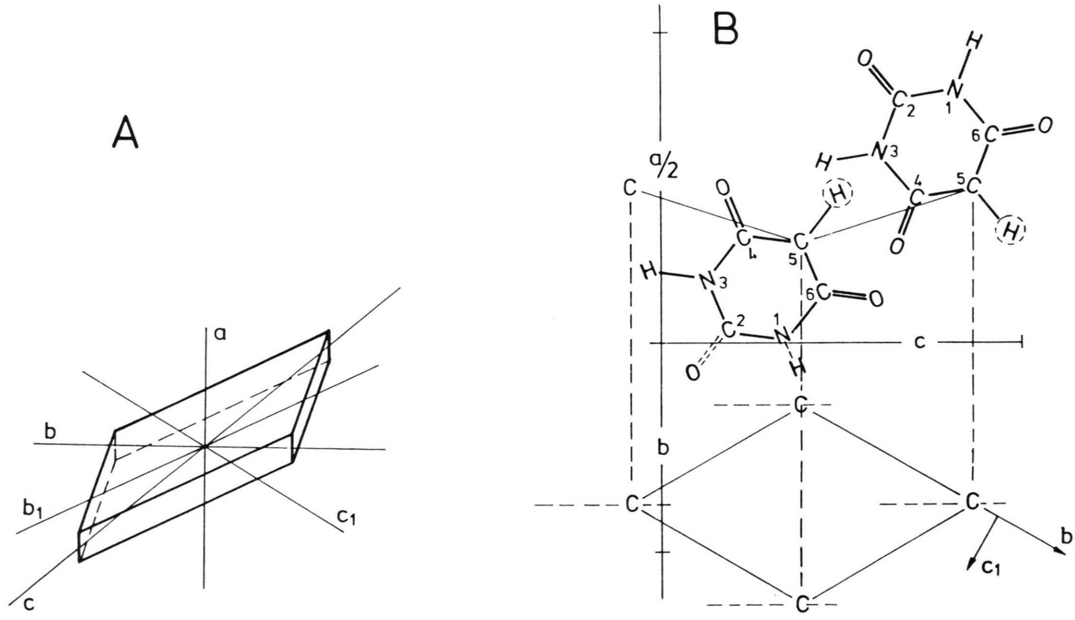

Fig. 3. Crystal morphology (A) and molecular structure of unit cell in single crystals (B) of barbituric acid [2].

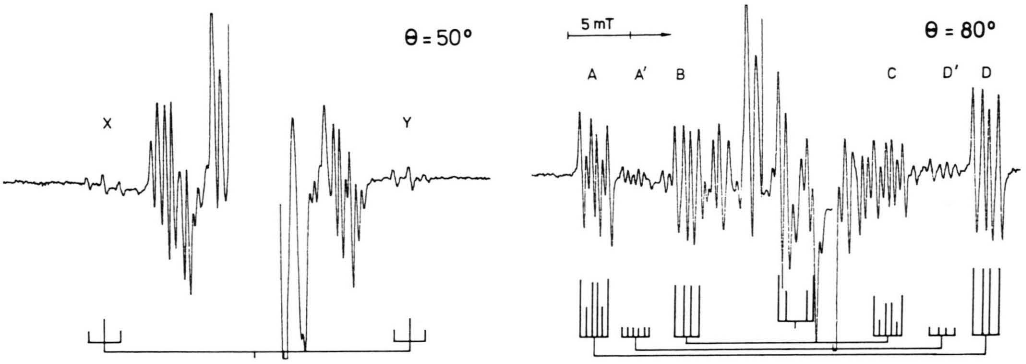

Fig. 4. ESR spectra of barbituric acid in two different orientations within the $ab_1$-plane at 77 K. Microwave frequency: 9.32 GHz, power level: 1.26 mW.

## Experimental

### *Radical production*

Single crystals of barbituric acid were grown from saturated aqueous solutions by slow evaporation at room temperature [1, 2]. Radicals were generated by exposure to X-rays from a 100 kV tube at 77 K. The dose applied was 70 kGy.

### *Crystal structure*

Single crystals of barbituric acid are orthorhombic with space group $P_n2_1a$ [10]. The unit cell contains four molecules of barbituric acid and two molecules of water [2, 10]. The crystal structure is shown in Fig. 3 [2].

### *Level-anticrossing effect (LAC)*

The exchange energy can be measured by observing the LAC effect using an ESR spectrometer. It occurs at the magnetic field strength where the singlet state (S = 0) is crossed by one of the triplet Zeeman levels (S = 1) as shown in Fig. 2 [3, 4]. When this happens the intensity of one of the two fine structure transitions appears to vanish. The exchange energy is given by [3]

$$J = -h\nu - \frac{2}{3} D \text{ for } J < 0. \qquad (4)$$

When the sign of $D$ is known the sign of $J$ can be deduced depending on the location of the effect on the low or high field side of the monoradical spectrum. The LAC range is reached either by temperature variation following Eqn. (3) or by variation of the microwave frequency ν.

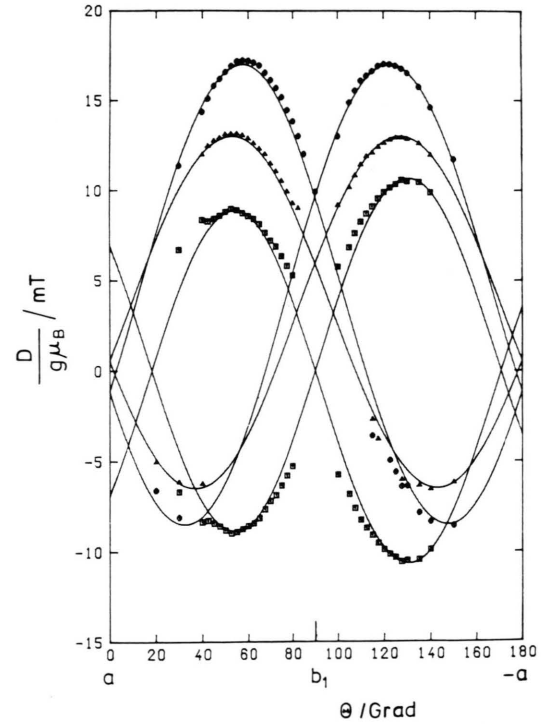

Fig. 5. Fine structure splitting constant of radical pairs AD (●), A′D′ (▲) and BC (■) in barbituric acid marked in Fig. 4 *versus* angle of rotation in the $ab_1$-plane. Microwave frequency: 9.32 GHz, power level: 1.26 mW. Full lines have been calculated from Eqn. (1a).

### *ESR measurements at fixed frequencies*

ESR measurements at 77 K were performed working at X-band (Bruker ER-220 D LR) and at Q-band (Varian E9). The first derivative of microwave absorption against magnetic field strength was registered.

### *ESR measurements with variable frequencies*

The ESR spectrometer described by Knopp [3] utilizing variable frequencies between 8 GHz and 12 GHz including an adjustable cylindrical cavity in the TE(011) mode was also used for measurements.

## Results

### *Monoradical and radical pairs at 77 K*

In Fig. 4 ESR spectra registered at 77 K with the magnetic field in two different orientations parallel to the $ab_1$-plane are presented. Lines of several different pairs are observed showing hyperfine splittings of two α-protons [2]. These are spaced at equal distances from the lines of the monoradical. The latter are characterized by hyperfine interaction with a single proton [1].

Fig. 5 shows the parameter $D$ of the fine structure splitting in dependence on crystal orientation in the magnetic field. Pairs AD and BC were already reported by Melø *et al.* [2]. These are characterized by the same hyperfine structure. According to Melø *et al.* the two types of pairs differ only in orientation of the vector linking the paired radicals, *i.e.* by site splitting. Hence AD and BC should show the same fine structure. However, in our experiments shown in Fig. 5 the pair BC did not display the orientational variation expected. Therefore BC should not be regarded as the same pair as AD differing not only by an angle of rotation but also in the orientation of the linking vector relative to the molecular planes of the paired radicals. Therefore the linking vector of the pair BC is not situated in the $ab_1$-plane as that of the pair AD. In addition, the distance of the paired radicals is substantially longer giving a smaller maximum splitting. The values of the fine structure constants of AD at 77 K are $D = -43.5$ mT, and $E = -0.4$ mT. The resulting distance is $R = 0.58$ nm [2].

In our work two more pairs labelled A′D′ and XY were seen (Fig. 4). A′D′ is visible at angles between $\Theta = 60°$ and 120°. At 77 K the intensity relation of A/A′ or D/D′ is about 4:1. At this orientation the fine structure splitting Δ of A′D′ is smaller than that of AD. The hyperfine splittings of A′D′ are the same as those of pairs AD and BC showing a quartet (Fig. 4, right). Some additional satellite lines are visible. These are attributed to forbidden transitions involving the nuclear Zeeman term [11, 12] since they are absent in Q-band spectra.

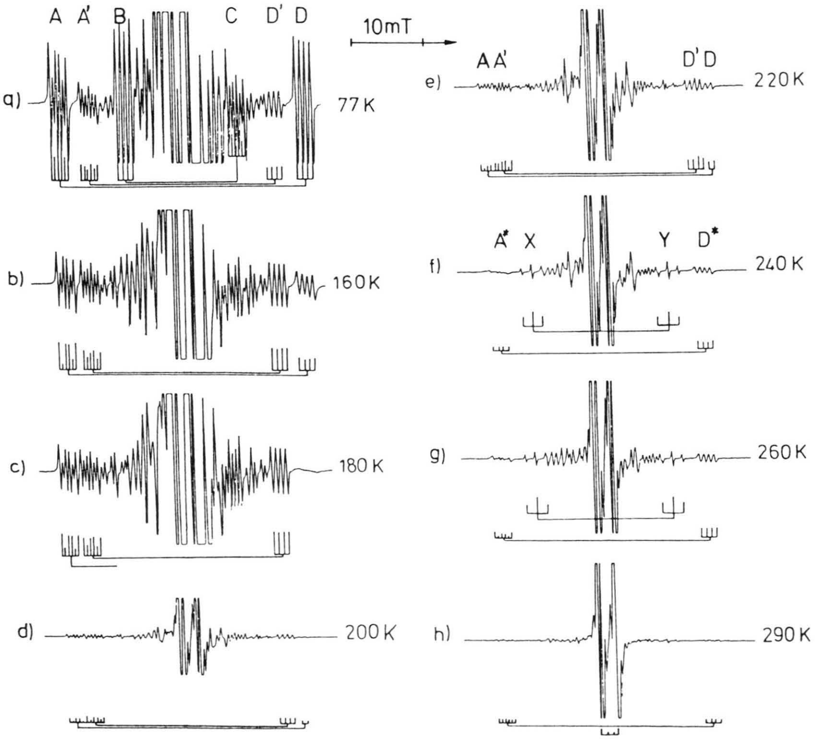

Fig. 6. ESR spectra of barbituric acid *versus* temperature in a fixed orientation with $\Theta = 80°$ within the $ab_1$-plane. Microwave frequency: 9.33 GHz, power level: 1.26 mW.

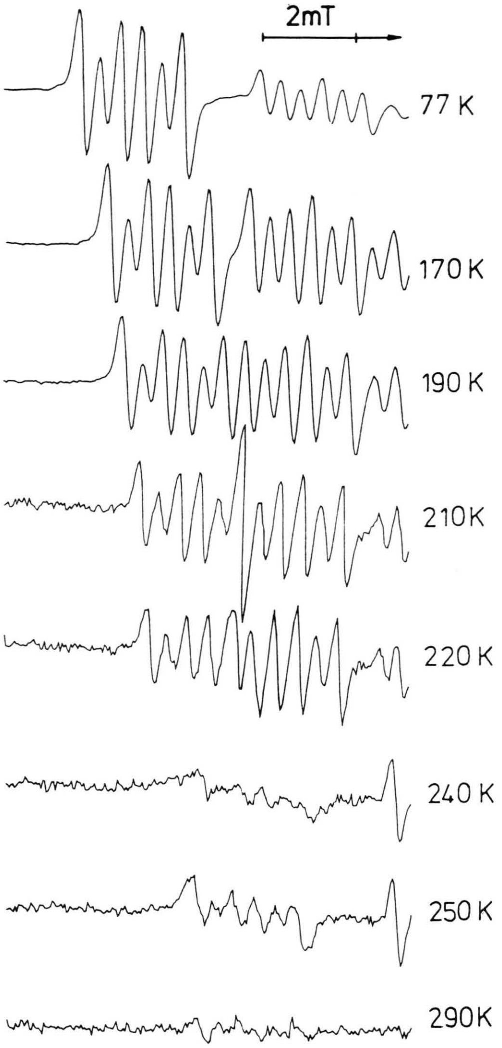

Fig. 7. Line groups A and A′ of ESR spectra of barbituric acid *versus* temperature under identical conditions are those of Fig. 6.

The other new pair XY is seen at $\Theta = 50°$ (Fig. 4, left). The hyperfine splitting in this orientation is composed of a 1:2:1 triplet.

### *Temperature-dependent ESR measurements in the range of 77 K to 300 K*

Radical pairs in barbituric acid are stable at 77 K as shown in Fig. 6. The intensity of all lines is decreased by warming up with exception of A′ in the temperature range from 77 K to 190 K. Atomic hydrogen atoms acquire increased mobility and may recombine with barbituric acid radicals. Up to 290 K radical pairs are still visible (Fig. 6). Refrigeration to 77 K does not increase the remaining absorption.

### *Thermal shift of line groups*

Parts of the spectra shown in Fig. 6 representing lines A and A′ are repeated in Fig. 7 with increased spread. Between 77 K and 190 K the intensity of A decreases while that of A′ increases. Correspondingly, the intensity relation changes from 4:1 at 77 K to about 1:1 at 190 K returning to 4:1 by recooling to 77 K. This behaviour is likely to result from different relaxation mechanisms of pairs AD and A′D′. Different saturation behaviour at 77 K has indeed been found for both groups [13].

Between 77 K and 230 K line groups A and A′ just as D and D′ are approaching each other and finally merge. This reversible process is slow up to 230 K but discontinuous at 235 K. At this temperature the combined pair A*D* appears. Between 235 K and 290 K no further shift is seen.

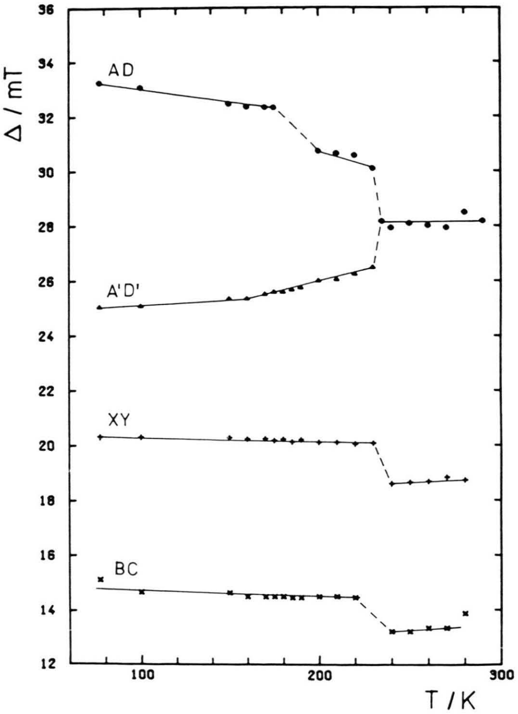

Fig. 8. Fine structure splitting *versus* temperature of pairs of line groups attributed to radical pairs. Microwave frequency: 9.33 GHz, power level: 1.26 mW. Orientation: $\Theta = 80°$ within the $ab_1$-plane.

The absorption lines BC and XY show a similar behaviour, a slow shift from 77 K to 230 K, a jump at 235 K and again a slow shift up to 290 K (Fig. 8).

Leaving aside the region of the LAC effect the thermal shifts between 77 K and 230 K as well as those between 240 K and 290 K can be described by Eqn. (2) as shown in Table I. The values of $\gamma$ are small and of the same order of magnitude as in KHM [14]. The sign of the temperature coefficient is negative for pairs AD, BC and XY below 235 K and positive or zero at higher temperatures. For the pair A′D′ $\gamma$ is positive below 235 K and zero after the conversion to pair A*D* above 240 K.

## Discussion

In order to explain the reversible transformation of AD and A′D′ into A*D* and the discontinuous change of the fine structure constant of all pairs the following assumptions are made.

1. The pyrimidine rings of the radicals are not oriented exactly parallel to the ac-plane, but are slightly tilted. This results in slightly deviating distances of paired electrons of different pairs resulting in different fine structure constants as measured for pairs AD and A′D′.
2. The orientation of individual radicals is changing with temperature due to a change of tilt angle. This angle decreases with rising temperature leading to the observed approach between splittings of pairs AD and A′D′.
3. At 235 K all radicals switch into a symmetrical orientation with respect to the ac-plane resulting in equally distant electrons of pairs AD and A′D′ and a concomitant jump for all other pairs at the same temperature. This change may constitute a phase transition.

Line groups XY are attributed to a different type of pair. Its distance vector is assumed to be parallel to the *b*-axis since little anisotropy was seen when the magnetic field was rotated in the ab$_1$-plane. However, the lines XY were visible in a fairly small segment only. The assignment is supported by the value derived for the effective distance of the pair which is

Table I. Temperature dependence of fine structure splitting $\Delta$ of radical pairs of barbituric acid.

| Radical pair | $\Delta_0$* mT | Temperature range $T/K$ | $\frac{\delta\Delta}{\delta T} / \frac{10^{-3}\ \mathrm{mT}}{K}$ | $\frac{\gamma}{10^{-4}\ K^{-1}}$ |
|---|---|---|---|---|
| *ab*$_1$-plane, $\Theta = 52.5°$ | | | | |
| AD | $33.3 \pm 0.1$ | $77 \leq T \leq 175$ | $-(7 \pm 1)$ | $-(2.1 \pm 0.2)$ |
| | | $195 \leq T \leq 230$ | $-(19 \pm 2)$ | $-(5.7 \pm 0.4)$ |
| | | $230 \leq T \leq 235$ | – | – |
| A*D* | | $235 \leq T \leq 300$ | $0 \pm 5$ | $0.0 \pm 1.0$ |
| A′D′ | $25.1 \pm 0.1$ | $77 \leq T \leq 160$ | $5 \pm 1$ | $2.0 \pm 0.4$ |
| | | $160 \leq T \leq 230$ | $17 \pm 2$ | $6.8 \pm 0.8$ |
| | | $230 \leq T \leq 235$ | – | – |
| A*D* | | $235 \leq T \leq 300$ | $0 \pm 5$ | $0.0 \pm 1.0$ |
| *ab*$_1$-plane, $\Theta = 120°$ | | | | |
| AD | $33.8 \pm 0.1$ | $77 \leq T \leq 210$ | $-(9 \pm 2)$ | $-(2.7 \pm 0.7)$ |
| | | $210 \leq T \leq 230$ | $-(22 \pm 4)$ | $-(6.5 \pm 1.2)$ |
| | | $230 \leq T \leq 250$ | – | – |
| A*D* | | $250 \leq T \leq 270$ | $0 \pm 5$ | $0.0 \pm 1.7$ |
| A′D′ | $25.3 \pm 0.1$ | $77 \leq T \leq 190$ | $5 \pm 1$ | $2.0 \pm 0.4$ |
| | | $190 \leq T \leq 230$ | $16 \pm 1$ | $6.3 \pm 0.4$ |
| | | $230 \leq T \leq 250$ | – | |
| A*D* | | $250 \leq T \leq 270$ | $0 \pm 5$ | $0.0 \pm 1.0$ |
| *ab*$_1$-plane, $\Theta = 80°$ | | | | |
| XY | $20.3 \pm 0.1$ | $77 \leq T \leq 230$ | $-(1.5 \pm 0.5)$ | $-(0.8 \pm 0.3)$ |
| | | $230 \leq T \leq 240$ | – | – |
| | | $240 \leq T \leq 300$ | $5 \pm 1$ | $2.5 \pm 0.5$ |
| BC | $21.2 \pm 0.1$ | $77 \leq T \leq 220$ | $-(2.2 \pm 1.0)$ | $-(1.1 \pm 0.5)$ |
| | | $220 \leq T \leq 240$ | – | |
| | | $240 \leq T \leq 300$ | $4 \pm 1$ | $1.9 \pm 0.6$ |

* $\Delta_0 = \Delta(77\ \mathrm{K})$.

0.62 nm at 77 K. A value of 0.62 nm at 300 K is given by the crystallographic determination of Jeffrey *et al.* [9].

The temperature coefficient for XY is the smallest of all. Thermal expansion is expected to be minimal parallel to the *b*-axis which is normal to the pyrimidine rings.

The hyperfine structure of XY is due to two equivalent protons. The CH-bonds of radicals stacked perpendicular to the pyrimidine plane are parallel and hence result in equivalent splittings.

### *The LAC effect*

At (180 ± 10) K the intensity of the high field absorption designated $D$ decreases while the corresponding absorption $A$ remains unchanged. Above 190 K the intensity is recovering (Fig. 9). $D$ remains visible up to 235 K where $D$ merges with $D'$ to give $D^*$. The LAC effect is absent in the Q-band in the same range of temperatures. As $D < 0$ [2] the LAC effect appears on the high field side only when $J$ is negative. From Eqn. (4) the exchange energy at (180 ± 10) K is $J/h = -(9.6 \pm 0.5)$ GHz. The exchange energy at 77 K can be derived from a linear extrapolation of transition temperatures at different frequencies. Additional measurements were performed at 11.0 GHz and 11.6 GHz (Fig. 10). A value of $-(15.1 \pm 0.6)$ GHz is found for $J/h$ at 77 K (Table II). The corresponding temperature coefficient is $\beta = -(3.8 \pm 0.8) \times 10^{-3}\mathrm{K}^{-1}$. It is seen from Table II that the exchange energy of barbituric acid has the highest value. This observation is in accord with the conclusion from earlier work attributing an increased thermal stability to pairs with higher exchange energy [3].

The "bleaching" of radical pairs after irradiation of a single crystal of 1-MU with UV light, was described by Flossmann *et al.* [4]. In our work single crystals of barbituric acid were exposed to an intense beam of a mercury-high pressure lamp with wave-lengths higher than 300 nm. Even after irradiation of 1 h no bleaching occurred. The same lines were observed before and after irradiation.

The results of variable temperature measurements in this work and in the papers of Knopp [3] and Flossmann *et al.* [4] clearly show a relation between stability against increasing temperature and UV light as well as the value of the exchange energy. A theoretical treatment of the dependence of the ex-

Table II. Exchange energy $J$(77 K) and temperature coefficient β of exchange energy (Eqn. (3)) at radical pairs in KHM, 1-MU and barbituric acid.

| Crystal | Exchange energy ($J$(77 K)/h) GHz | Temperature-coefficient $\beta/(10^{-3}\,K^{-1})$ | Reference |
|---|---|---|---|
| KHM | $+(12.3 \pm 0.3)$ | $+(3.2 \pm 0.5)$ | [3] |
| 1-MU | $-(10.06 \pm 0.01)$ | $-(3.1 \pm 0.1)$ | [3], [4] |
| barbituric acid | $-(15.1 \pm 0.6)$ | $-(3.8 \pm 0.8)$ | – |

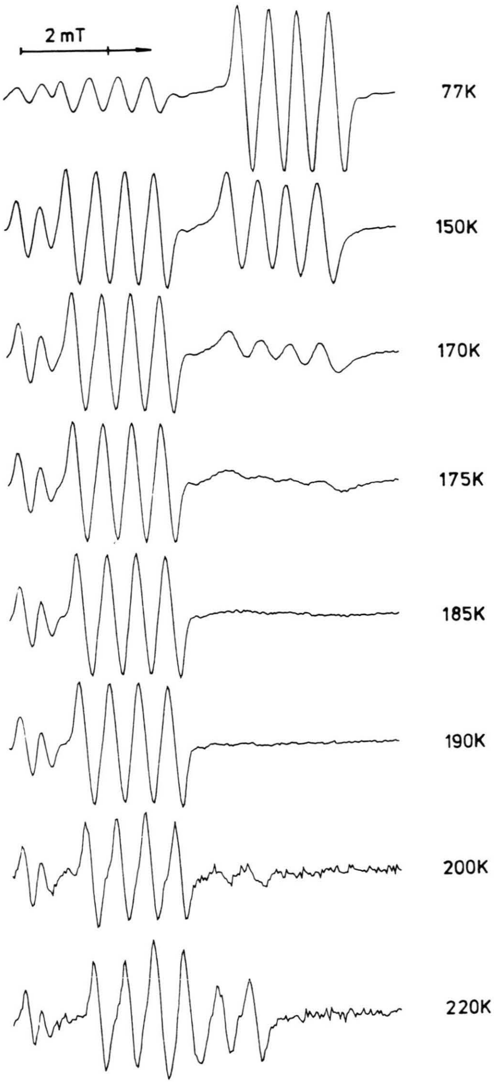

Fig. 9: Variation of line groups D and D′ with temperature attributed to level anticrossing (LAC). Conditions are those of Fig. 8.

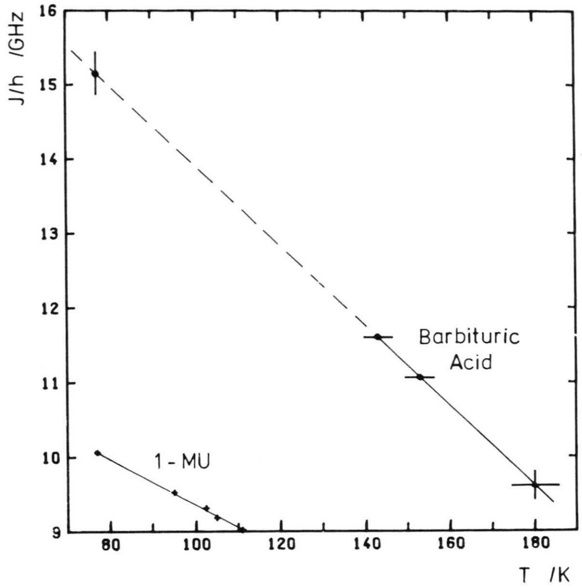

Fig. 10: Temperature dependence of exchange energy $J$ for radical pairs in barbituric acid and in 1-methyl uracil [3, 8].

change energy $J$ with the stability of radicals against temperature and UV light has not been advanced as yet.

## Conclusions

In single crystals of barbituric acid·2 $H_2O$ radical pairs A′D′ and XY have been detected in addition to pairs AD and BC that were known before. In contrast to earlier work [2] the pair BC must be regarded as different from pair AD not only with respect to orientation but also in mutual distance. Exact determinations of the principal values of the fine structure tensors can be derived from orientation-dependent measurements in several planes of the crystal. All pairs are composed of the same type of monoradicals lacking a hydrogen atom at C5. Pairs AD and A′D′ are attributed to the same type of pair. AD and BC differ in distance vectors while AD and A′D′ cannot be distinguished above 230 K forming a common pair A*D*. This is again split at lower temperatures into pairs AD and A′D′. The reversible phenomenon may be caused by a phase transition of the crystal structure. The observed temperature variation of the fine structure splittings is linear in limited ranges.

The pair AD is the only one for which a level anticrossing (LAC) effect has been found in our experiments. The associated exchange energy $J$ was measured at various frequencies yielding an extrapolated value of $J/h = -(15.1 \pm 0.6)$ GHz at 77 K.

The distance vector of pair XY is oriented along the crystallographic $b$-axis at right angles to the molecular ring planes. Its length is 0.62 nm at 77 K which is larger than the distance of all other pairs.

### *Acknowledgements*

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