Electron Paramagnetic Resonance Study of Thermal Decomposition of Molecules. I. Barbituric Acid Derivatives[1]

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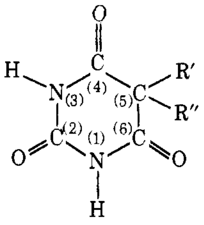
Abstract: Free radicals in the heat-treated barbituric acid derivatives were studied by epr spectroscopy. The radicals were identified in alloxan, uramil, violuric acid, dilituric acid, and cyclobarbital. Three types of decomposition were observed: (a) loss of the substituent, (b) change in the substituent, and (c) scission of the ring. The CO–NH_2 fragments were positively identified in the decomposition process of alloxan and violuric acid.

Derivatives of barbituric acids, often called "barbiturates," make a well-known group of pharmacologically interesting compounds. Interest in these compounds arises from the fact that some of them have hypnotic properties. The depressant action of the barbituric acid derivatives varies from mild sedation to anesthesia, depending upon the particular barbiturate selected and upon the dose and way of administration. In fact, only a small number of the known barbituric acid derivatives exhibit appreciable therapeutic properties.

As a class, barbituric acids are not very stable. They undergo decomposition both in solution and solid. The hydrolytic decomposition in solution starts by cleavage of the pyrimidine ring.[2] Solid samples must decompose in a different way. In some cases free radicals as the intermediary products of decomposition can be observed. The aim of the present study is to identify such radicals by electron paramagnetic resonance (epr) and thus learn somewhat about the pathway of decomposition.

Experimental Section

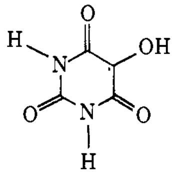
A number of barbituric acid derivatives of general formula I or some other tautomeric form were studied. The compounds were prepared in this Institute or obtained from various commercial sources. The specimens in powdered form after recrystallization or without further purification were evacuated and sealed. They were heat-treated by storage at some elevated temperature and then tested by epr spectroscopy. Alternatively, the samples were placed in the spectrometer cavity and gradually heated with the simultaneous recording of the spectra. In such a way relatively short-lived radicals could also be observed. In this study a Varian E-3 spectrometer was employed.



I

Structure of Radicals

Alloxan. Alloxan is usually found with a molecule of bound water, namely as a compound of general structure I with R′ = R″ = OH.[3] When the compound is kept at about 160° under evacuation, most of the alloxan molecules loose water. However, a fraction of the molecules decompose by that treatment, and free radicals as the first step product are formed. The first derivative epr spectrum of these radicals is represented by the top curve in Figure 1. By comparison of this curve with the spectra of the radicals produced by other means in crystalline alloxan,[4,5] one easily recognizes the radicals of structure II, formed from alloxan by a loss of a hydroxyl group.



II

Different epr signals are observed when alloxan is heated above 170° in the spectrometer cavity. The pattern consists of five sharp lines of the intensity ratio of approximately 1:3:4:3:1, as demonstrated by the middle curve in Figure 1. The separation between the lines is about 7.2 G. These resonance characteristics suggest that the unpaired electron interacts with two nuclei having spin $I = {}^1/_2$ (protons) and a nucleus with spin $I = 1$ (nitrogen). Such a pattern should account for radicals III. In order to verify this assignment,

$$R\text{—}\dot{N}H_2$$

III

completely deuterated alloxan was prepared by recrystallization from heavy water. The decomposition product of the deuterated compound must be R-ND_2. Since the deuteron coupling is about 6.5 times smaller than the proton coupling, the epr spectrum of this radical must be essentially a triplet of the intensity ratio 1:1:1. The lowest curve in Figure 1 proves that that is really the case. The middle line is always found a little larger than the outer two, probably because some additional resonance is present at that place.

From the spectra of nondeuterated and deuterated radicals the following coupling parameters can be deduced: $A_H = A_N = 7.2 \pm 0.3$ G. Both of the couplings are expected to be approximately proportional to the spin density on nitrogen, the constants of proportionality being about 28[6a] and 31[6b] G for nitrogen

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[1] This work was supported by the Research Fund of Croatia.

[2] W. J. Doran in "Medicinal Chemistry," Vol. 4, E. F. Blicke and R. H. Cox, Ed., Wiley, New York, N. Y., 1959.

[3] J. F. Thorpe and M. A. Whiteley, "Thorpe's Dictionary of Applied Chemistry," Vol. 1, 4th ed, Longmans, Green and Co., London, 1947, p 243.

[4] M. Kashiwagi, *J. Mol. Spectrosc.*, 20, 190 (1966).

[5] J. N. Herak, *J. Amer. Chem. Soc.*, 91, 5171 (1969).

[6] (a) C. L. Talcott and R. J. Myers, *Mol. Phys.*, 12, 549 (1967); (b) H. W. Shields, P. J. Hamrick, Jr., and W. Redwine, *J. Chem. Phys.*, 46, 2510 (1967).

Figure 1. First derivative epr spectra of alloxan, recorded after heating the specimen at 160° (upper curve) and upon heating at about 180° (two lower curves); the bottom spectrum belongs to the deuterated compound.

and proton, respectively. Sometimes, as a consequence of the spin delocalization over the neighboring atoms, different values are quoted. Thus, the equivalence of the nitrogen and proton couplings is not unexpected.

The lifetime of the R–NH_2 radicals is of the order of seconds. One actually observes the stationary concentration of these radicals as they are formed and disappear in the decomposition process. If the decomposition rate is slow, the stationary concentration is low and the radicals might not be observed at all. The decomposition takes place in melt and that is the reason that one observes only the isotropic component of the proton and ^{14}N couplings.

Uramil. Uramil (5-aminobarbituric acid) may be considered to be structure I with $R' = H$, $R'' = NH_2$. Upon heating the compound the radicals represented by the upper curves in Figure 2 are formed. Most of the radicals present are obviously of the same type as those in alloxan (compare the top curves in Figures 1 and 2). However, it is not seen how radicals II can be formed by decomposition of this compound. It is possible that we are dealing with the radicals $>C_6$–OH, which might be formed from another tautomeric form of the compound by abstraction of the hydrogen atom. In that case imino rather than amino group is bound on C_5.

Violuric Acid. This compound is not exactly of structure I. Instead of two groups, R′ and R″, the oximino group, =NOH, is present.[7]

After heating violuric acid at about 100°, radicals are observed. Their formation is associated with the loss of water of crystallization and hence with decomposition of the crystal structure. By that procedure some molecules decompose and free radicals as the

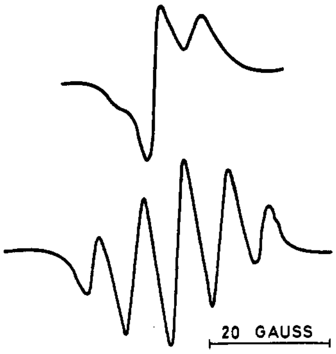

Figure 2. Epr spectra of uramil heated at about 200° (upper curve) and violuric acid heated at about 240° (lower curve).

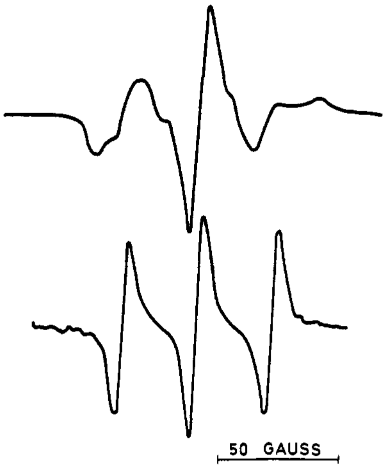

Figure 3. The derivative epr absorption curves of dilituric acid in solid (upper tracing) and melt (lower tracing).

primary decomposition products are formed. These radicals could not be positively identified.

Different radicals are formed when violuric acid is heated in the spectrometer cavity at or above the decomposition temperature (240°). The observed five-line epr pattern, as shown by the lower tracing in Figure 2, is the same as the high-temperature pattern of alloxan. Hence, in violuric acid too, radicals III are formed.

Dilituric Acid. This barbituric acid derivative, having $R' = H$, $R' = NO_2$, decomposes readily upon heating. Radicals are observed in the samples stored for longer time even at room temperature. A typical epr signal found is shown in Figure 3 (upper curve). It is a typical powder pattern of the axially symmetric ^{14}N coupling with the axially symmetric g tensor.[8] From this resonance structure it is possible to determine the principal elements of both the hyperfine splitting tensor and g tensor. One finds $A_{\parallel} = 44$, $A_{\perp} = 25$ G. The unpaired electron couples with an ^{14}N nucleus either from the pyrimidine ring or from the nitro group. Very similar tensor parameters have been observed in the irradiated single crystal of 5-nitrouracil[9] and interpreted to be associated with the unpaired electron in the σ orbital of the ring nitrogen, N_1. We are inclined to believe that in the present case the hyperfine splitting comes from the nitro group

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(7) Reference 3, p 626.

(8) P. W. Atkins and M. C. R. Symons, "The Structure of Inorganic Radicals," Elsevier, Amsterdam, 1967, p 271.

(9) B. Benson and W. Snipes, *Mol. Phys.*, **20**, 357 (1971).

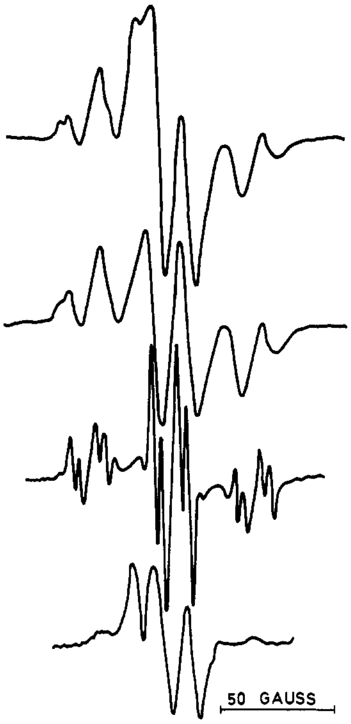
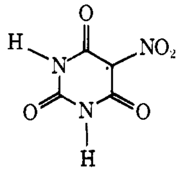


Figure 4. Epr spectra of cyclobarbital. The top and the bottom curves refer to the specimen heated below and above 90°, respectively. The middle curves refer to the γ-irradiated powder and single crystal, respectively.

nitrogen atoms. The reasons are as follows. (a) Radicals in dilituric acid can be produced upon a very mild treatment (like prolonged storage at room temperature). That indicates that very low energy is required for the radical formation. For abstraction of a hydrogen atom from either N_1 or C_5 the energy of several electron volts is needed. If the abstraction of the C_5 hydrogen takes place, the π electrons of the nitro group conjugate with the ring π electrons, and thus this additional resonance energy compensates for the loss of energy associated with the release of the hydrogen atom. In contrast, release of the hydrogen from a ring nitrogen, especially if the unpaired electron remains in the nitrogen σ orbital, is highly improbable, since there is no additional resonance to compensate for the energy loss upon the scission of the N–H bond. The π-electron radical formed by a loss of the N–H hydrogen is also ruled out, because the observed coupling parameters, particularly $A_\perp$, are too high for such a radical. (b) In all the barbituric acid derivatives studied in the present work, and also in about ten compounds of structure I exposed to small reactive species,[10] changes at the substituents, R′ or R″, take place. Dilituric acid is expected to behave in the same way. Thus, the epr spectrum of heat-treated dilituric acid (the compound treated in other ways gives the same resonance pattern[10]) probably accounts for radicals associated with the NO_2 group.

We believe that we are dealing with radical IV. The observed large nitrogen coupling is in a good agreement with the expectations for the NO_2 group.[11] The large $A_\perp$ value is also not in contradiction with other radicals containing nitro groups in solid.[12] However, in order to understand better this behavior, further study is needed.



IV

Radicals IV are detectable even in the melt, in which the molecular motion averages out the anisotropic component of the hyperfine splitting and a regular 1:1:1 triplet is observed (see lower curve in Figure 3). From this spectrum the isotropic coupling $A_N = 31$ G is deduced; it is the same as determined from the spectrum in solid: $A_N = (1/3)(2A_\perp + A_\parallel) = 31$ G.

Cyclobarbital. Cyclobarbital has 1-cyclohexenyl and ethyl groups substituted at position 5 in structure I. Upon heating three types of radicals are observed. One of them is represented by a singlet, often observed even in the untreated specimen. The nature of these radicals remains unknown. When the compound is heated at 90°, new radicals of different kind are formed. They are represented by the six-line epr pattern as shown in Figure 4 (top curve). Interpretation of this resonance structure is also not very easy because the intensity ratio and separation between the lines does not fit to any scheme. However, we found that essentially the same pattern is observed in γ-irradiated cyclobarbital, as shown by the second curve in Figure 4. The upper two curves in Figure 4 differ only in the already mentioned additional singlet presented in the heat-treated sample. Thus, the radicals formed by thermal decomposition of the compound can also be studied by investigating the radicals in the γ-irradiated single crystal of cyclobarbital.

A typical single-crystal epr pattern of cyclobarbital is presented in Figure 4 (third curve). The dominant resonance structure consists of three doublets further split by a small proton coupling. The middle lines seem to be much larger than the others. The single crystal analysis shows that they are twice as large as the others, but they are at the position of other strong resonance absorption belonging to other radicals. The well-resolved pattern is produced by coupling of four protons with the unpaired electron. Two of the protons are equivalent β protons with isotropic coupling of 33.5 G. The remaining two are more anisotropic α protons. The pincipal values of the couplings are $A_1^{(1)} = 19.5$, $A_2^{(1)} = 11.5$, and $A_3^{(1)} = 6.5$ G, and $A_1^{(2)} = 5.5$, $A_2^{(2)} = 3.0$, and $A_3^{(2)} = 1.0$ G. The direction cosines could not be determined with any reasonable precision, because obviously there exists more than one magnetically distinct molecule in the unit cell. The site splitting is rather small and induces only broadening of the lines. Consequently, one is able to measure only the "average" couplings for at least two molecules. In such a case $A^2(\theta)$ is not a sinusoidal curve. That introduces errors in the determination of the principal elements of the coupling tensors. The direction cosines are very much affected by the choice of the off-diagonal elements of the A_{ij}^2 matrix. It is not the case with the principal elements: the above cou-

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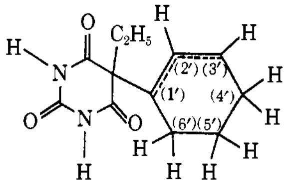
(10) J. N. Herak and J. J. Herak, submitted for publication.

(11) P. H. Rieger and G. K. Fraenkel, *J. Chem. Phys.*, **39**, 609 (1963).

(12) W. M. Fox, J. M. Gross, and M. C. R. Symons, *J. Chem. Soc.*, 448 (1966).

plings are determined with the precision of ±1.5 and ±1 G for $H^{(1)}$ and $H^{(2)}$ protons, respectively.

The unpaired electron is undoubtly located in the cyclohexenyl ring. We conclude that the radicals observed must be of structure V. The unpaired spin is symmetrically distributed over the three unsaturated carbon atoms. The ring is not expected to be planar. Thus, the two equivalent β protons cannot be those on $C_{4'}$. We assume that the β protons are the axial hydrogen atoms on $C_{4'}$ and $C_{6'}$; the equatorial ones are not observed.



V

The larger α-proton coupling, with the isotropic component of 12.5 G, comes from the spin density on $C_{3'}$, and judging from the β-proton couplings we assume that the same spin density must be on $C_{1'}$. The small α-proton coupling having an isotropic component of 3.0 G is associated with the $C_{2'}H$ hydrogen. According to the McConnell relation[13]

$$A_H = Q_\alpha \rho_c$$

with $Q_\alpha = 26$ G,[14] one gets $\rho_{3'} = 0.48$ and $\rho_{2'} = 0.12$ and sees that essentially all the spin density is located in the $C_{1'}$–$C_{3'}$ region. It is possible that the density on $C_{2'}$ is negative.

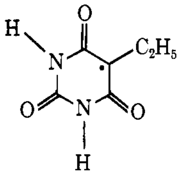
The β-proton couplings depend upon the angle, θ, between the C_β–H bond and the direction of the π orbital of the electron as[15]

$$A_H{}^\beta = Q_\beta \rho_c \cos^2 \theta$$

where Q_β is a constant of approximately 56 G.[14] Using $\rho_c = 0.48$ and assuming that for the axial hydrogens $\theta = 0°$, one expects β couplings of 27 G. That is in fairly good agreement with the observations. The somewhat larger observed value can be understood if we deal with the rocking conformation of the $\cdot C{-}CH_2$ fragment, as discussed earlier.[16]

A different type of radicals is detected after further heating the compound. Radicals V disappear and predominantly a 1:2:1 triplet remains. Although from the powder spectra one cannot be sure, there are good reasons to believe that this spectrum belongs to radical VI. The proton couplings of 11 G are the same as in that radical produced in the single crystal of barbital by ionizing radiation.[17]

Other Barbiturates. A number of other barbituric acid derivatives were also investigated, among others barbituric acid, barbital, phenobarbital, and hexobarbital. In these compounds either not very well-defined epr signals or no signals at all were observed. It is probable that the best experimental conditions were not found. It is also possible that the steady-state concentration of the radicals in the decomposition process was not high enough to be detected.



VI

Discussion

The structures of the detected radicals in the decomposition of the barbituric acid derivatives suggest that there are various ways of decomposition of these substances. In all the analyzed compounds a loss of the substituent group R′ or R′′ is detected. So, release of a hydrogen atom, hydroxy, amino, and cyclohexenyl groups has been undoubtedly proved. Similar conclusions were reached in the mass spectroscopy studies of barbituric acids.[18] The loss of R′ or R′′ is not necessarily the first step of decomposition for all the molecules of particular kind. Other competitive decomposition pathways are possible.

In cyclobarbital a change in the substituent is the predominant primary process. We believe that the loss of the hydrogen atom takes place at position 3′ in the cyclohexenyl substituent. It is not known whether this change is the first step in releasing of the cyclohexenyl group or whether radical VI is formed independently on the substituent group loss from C_5.

The existence of the R–NH_2 radicals during the decomposition process of alloxan and violuric acid is associated with the ring scission; we do not see any other possible way of formation of radicals III. Moreover, radicals III are probably the $\dot{C}O{-}NH_2$ species. Namely, if it were $-NH{-}CO{-}NH_2$ or something similar, we should be able to observe two nitrogen nuclei coupling with the unpaired electron, and that is in contrast with the observations. It is interesting to note that the same kinds of decomposition products are formed upon the electron impact in the mass spectroscopy experiments.[18] The source of the hydrogen atom that adds on nitrogen is either residual water, or it comes from the other NH fragment of the ring.

The present experiments demonstrate that by epr the structrue of some intermediary products in the thermal decomposition of molecules can be learned. Although it might not suffice for determination of the whole decomposition process, it may be of help in understanding at least some steps.

These experiments undoubtedly prove that in thermal decomposition of molecules free radicals may be found. This fact offers large possibilities to the electron paramagnetic resonance spectroscopy in the decomposition studies.

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