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ESR Study of the Reaction Sites in the Barbituric Acid Derivatives

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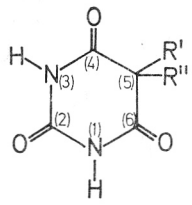
ESR spectroscopy has been used to analyse free radicals in the barbituric acid derivatives exposed to thermal H atoms or to excited Ar atoms. Reaction with both types of atoms leads to the same products, demonstrating that the most probable reaction sites in these molecules do not depend upon whether the *chemical* or *physical* type of energy is transferred to the molecules. In all the barbituric acid derivatives studied the reaction takes place at the 5-substituent of the compound. The reaction observed is either the loss of the substituent or a change in the substituent. The former reaction is observed in the pharmacologically inactive barbituric acid derivatives, the latter in the active ones.

INTRODUCTION

In the present study we want to find out the most reactive place in the barbituric acid derivatives when the *chemical* and the *physical* sort of energy is brought to these molecules. Instead of ionizing radiation, the much less energetic excited argon atoms were used as the source of the *physical* energy to be transferred to the molecules. The excited noble gas atoms as the reactive species were for the first time used by Ingalls and Young in studying the radicals in polymers[1].

EXPERIMENTAL

Various barbituric acid derivatives of structure I (or the same structure methylated on position 1) were obtained from commercial sources or were prepared in this Institute. The compounds were used after recrystallization or without further purifi-



I

cation. Powdered specimens were exposed to a beam of either thermal hydrogen atoms or excited argon atoms from a discharge of the relative gas. The exposure was performed in a special chamber described earlier[2]. The discharge was maintained by a radiofrequency generator. A magnet was used to deflect electrons before they could reach the specimen. We expect that the hydrogen atoms from the discharge have only thermal energies when they reach the specimen[3,4]. In such a way nothing

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but the *chemical* energy can be accounted for the damage introduced upon the contact of H atoms with the particular molecule.

In the discharge of argon various products are formed. In our experiment only long-lived active species could make reactions with the substances studied. The metastable Ar atoms in the $1s_3$ and $1s_5$ levels of the $3p^54s$ configuration are the obvious candidates. They have to survive several wall collisions before reaching the reaction site. An alternative source of the *physical* energy which is transferred to the molecules are the radiative $1s_2$ and $1s_4$ states of Ar. Although their natural life-time is much shorter than that of the metastables[5], it can be greatly increased as a result of the imprisonment of the resonance radiation[6]. Whatever the case, there is a transfer of energy from Ar* to the studied system, and consequent deexcitation of Ar. The transferred energy of about 11.6 eV is high enough to break any chemical bond of the molecules under investigation. The exposure of the barbituric acid derivatives to both H and Ar atoms was performed at room temperature or at 77° *K*. After the exposure the samples were kept and investigated at the same temperature. In most cases the radicals were the same at both temperatures. In some compounds the low temperature spectra were not so well resolved as the ones at room temperature. In this paper the room temperature results will be discussed, except when otherwise mentioned.

The radicals as the primary reaction products were analysed by a Varian E-3 ESR spectrometer.

IDENTIFICATION OF REACTION PRODUCTS

Barbituric Acid

The room temperature exposition of barbituric acid (compound I, $R' = R'' = H$) to both H and Ar atoms leads to the same radicals. The radicals formed are represented by the top ESR curve in Fig. 1. The resonance structure

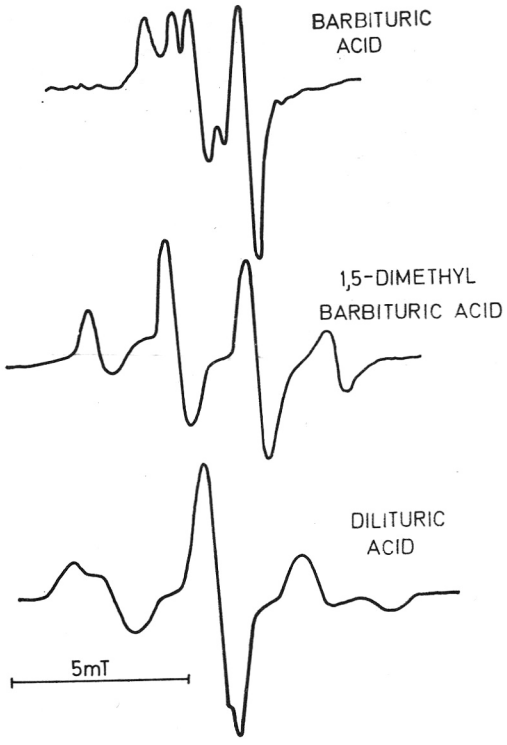
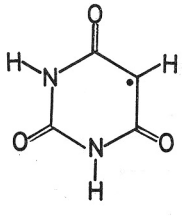
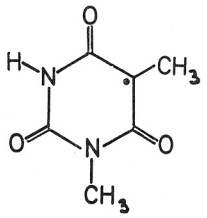


Fig. 1. First derivative ESR spectra of barbituric acid, 1,5-dimethyl barbituric acid, and dilituric acid after the exposure to H or Ar* atoms.

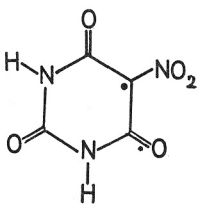
is not very clear. However, a close resemblance of this spectrum to that observed and successfully interpreted by the computer simulation for the randomly oriented radicals $CH(COO^-)_2$[7] and to the spectra of gamma-irradiated powdered barbituric acid[8], makes us believe that in the present experiment the same radicals are formed as upon irradiation. They are of structure II. In such a radical the α-proton coupling is anisotropic. In the irradiated compound the extreme coupling parameters were found to be close to 1 and 3 millitesla *(mT)*, respectively. In the present case approximately the same values can be deduced.



II



III



IV

1,5-Dimethyl barbituric Acid

1,5-Dimethyl barbituric acid (1-methylated compound I, R′ = H, R″ = CH_3) exposed to H atoms or to the excited Ar atoms showed a well resolved ESR quartet of the splitting $A/g\beta = 2.3$ *mT* and the line intensity ratio 1 : 3 : 3 : 1 (Fig. 1). There is no difference in the resonance spectra obtained in these two ways. Consequently, there is no difference in the radical species formed in the two different ways. The radicals are of structure III.

Dilituric Acid

It is very easy to produce radicals in dilituric acid (structure I, R′ = H, R″ = NO_2) both with H and Ar* atoms. In both cases the resonance patterns are the same (Fig. 1, lower tracing), and identical to that of the heat treated compound[9]. The principal values of the coupling tensor, $A^N_{||}/g\beta = (4.4 \pm 0.1)$ *mT* and $A^N_{\perp}/g\beta = (2.5 \pm 0.1)$ *mT*, and g-tensor, $g_{||} = 2.0022$ and $g_{\perp} = 2.0068$ with the limits or error of ±0.0005, are almost identical to the values in irradiated 5-nitrouracil[10]. These tensor elements rule out iminoxy radicals and π-electronic radicals. Although the spectroscopic data cannot rule out the σ-radicals proposed for 5-nitrouracil, the arguments stated earlier in the discussion of thermally generated radicals suggest that in dilituric acid we deal with radicals IV.

It should be noted that the compound exposed to a higher dose of H atoms exhibits broadening of the resonance lines. No sings of broadening could be noticed in the Ar* bombarded specimen. The different behaviour of the two cases must be due to the different ways of the radical production.

5-Ethyl barbituric Acid

Both regular 5-ethyl barbituric acid (I, R′ = H, R″ = CH_2CH_3) and the 1-methylated compound lose the hydrogen atom from C_5 upon exposure to H or Ar atoms. The resonance curve is shown in Fig. 2 (top curve). The hyperfine structure comes from two isotropic proton couplings of 0,97 and 1.48 *mT*,

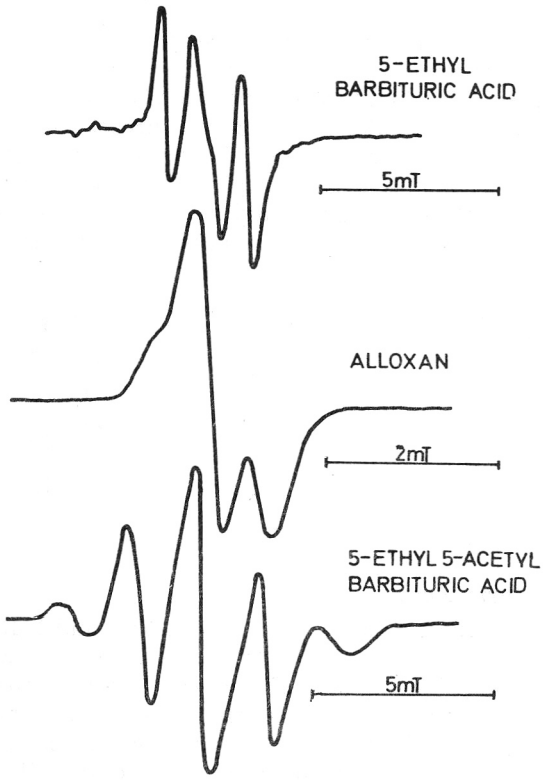


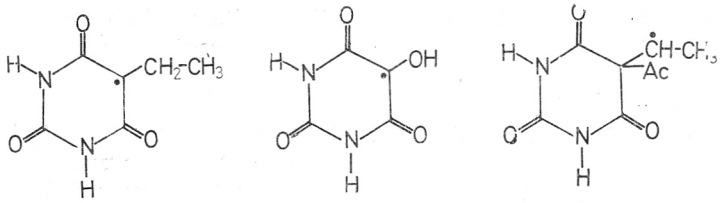
Fig. 2. ESR spectra of 5-ethyl barbituric acid, alloxan and 5-ethyl-5-acetyl barbituric acid, exposed to H or Ar* atoms.

respectively. They must be protons from the ethyl groups, belonging to radical V. These couplings are very similar to those found in the same radical in the gamma-irradiated single crystal of barbital[12]. The only difference is that in the present case the β-proton couplings are inequivalent. If one assumes the tetrahedral rotating conformation of the $—C^{\beta}H_2$-group then from the two β-proton couplings with the help of the relation

$$a_H^{\beta} = \varrho B \cdot \cos^2 \Theta$$

where ϱ is spin density on α carbon, B a constant and Θ angle between the $C^{\beta}—H$ bond and π orbital on C^{α}, one gets $\Theta_1 = 27^0$, $\Theta_2 = 44^0$.

Under certain conditions a small portion of radicals found in this substance have a more complicated resonance pattern. That is the same as in barbital, and will be discussed later.



Alloxan

When alloxan (I, R′ = R″ = OH) is exposed to the thermal H atoms radicals VI are formed[13]. The same resonance pattern and hence the same radicals are produced if the energy from excited Ar is transferred to the alloxan molecules (Fig. 2).

Uramil

Surprisingly, uramil (I, R′ = H, R″ = NH_2) exposed to the atoms from the discharge, either hydrogen or argon, exhibits essentially the same spectra as alloxan. One does not see how radicals VI can be made from the undamaged molecule. However, the actual tautomeric form of the molecule, and whether or not it binds a molecule of water like alloxan, is not known. It is also possible that in uramil the $>C_{(6)}$—OH radicals are formed.

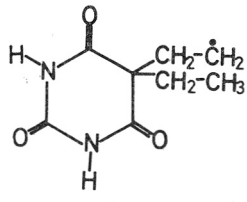
The same ESR spectra were observed in the heat-treated specimen[9].

5-Ethyl-5-acetyl barbituric Acid

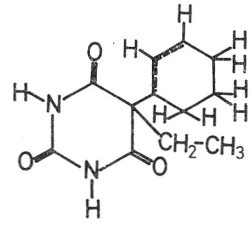
The reaction products of this substance with the thermal hydrogen atoms or excited argon are represented by the bottom curve in Fig. 2. In this spectrum one recognizes four equivalent proton couplings of 1.8 *mT*. The spectra are attributed to radical VII, formed by abstraction of a proton from the ethyl group.

Barbital

No difference in ESR spectra of barbital (I, R′ = R″ = CH_2CH_3) was observed when this substance was exposed to the thermal H atoms and to excited argon. Fig. 3 shows three typical resonance patterns of the radicals formed. The hyperfine structure seen in the top curve is due to four approximately equally coupling protons, the couplings being about 2.5 *mT*. This spectrum is similar to that of 5-ethyl-5-acetyl barbituric acid (Fig. 2), except that in the present case the couplings are much larger and a marked anisotropy of at least some of them is present. Consequently, the radicals in barbital cannot be of structure VII. We assume that radicals VIII account for this resonance pattern. Two equivalent α protons induce large anisotropy. Two equivalent β protons can be made inequivalent by cooling the system down to 77° *K*. At that temperature the β proton couplings are 2.15 and 3.30 *mT*, respectively. The combined anisotropic coupling of the two equivalent α protons leads to the significant broadening of the outer lines in the two top spectra in Fig. 3.



VIII



IX

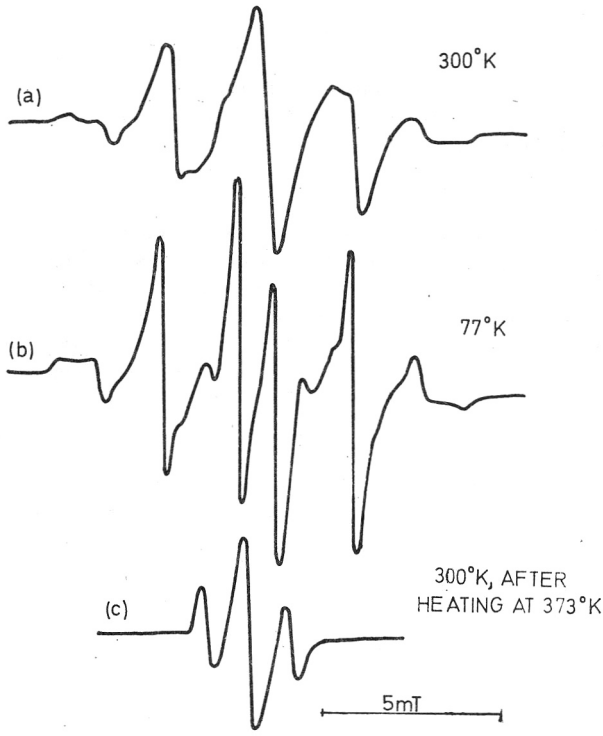


Fig. 3. ESR spectra of barbital after the exposure to Ar* atoms, recorded under three different conditions.

From the β-proton couplings, similar to the β-proton couplings in 5-ethyl barbituric acid, one gets $\theta_1 = 27^0$, $\theta_2 = 44^0$. However, in this case orientation of the $—C^{\beta}H_2$-group with respect to the ring remains unknown because orientation of the reference $—C^{\alpha}H_2$ group is not known.

Upon further warming the sample radicals VIII disappear and a small concentration of other radicals represented by the triplet resonance pattern (Fig. 3c) remains. These radicals are present in the specimen also before the heating but in much smaller concentration than radicals VIII. We believe that the triplet pattern belongs to radicals V, but in contrast to 5-ethyl barbituric acid, they have equivalent β-protons.

Cyclobarbital

The radicals in cyclobarbital (I, $R' = CH_2CH_3$, $R'' =$ cyclohexyl) formed in the present two ways are the same as those produced by gamma-irradiation or by heat-treatment[9]. The ESR pattern was earlier interpreted to belong to radicals IX, formed by a loss of a proton from the cyclohexenyl group.

DISCUSSION

Present experiments clearly demonstrate that in the barbituric acid derivatives the substituted groups on C-5 are the most labile sites in the molecules. In the compounds studied no injuries at other places were observed. It is interesting to note that practically the same radicals were formed upon

transfer of *physical* and *chemical* sort of energy to the compounds. Earlier[14], the selectivity in the radical production was found higher for H atoms than for ionizing radiation. In the present experiments the low energy excited atoms are as selective as the thermal hydrogen atoms.

In the barbituric acid derivatives two major types of damage were observed. It was either abstraction of the substituent on C-5, or a change in the substituent. Whenever one of the substituents was hydrogen, it could easily be abstracted by other H atoms from the discharge or it was released upon the energy transfer from Ar*. Also, when the change in the substituent took place, it was also abstraction of a hydrogen atom (barbital, cyclobarbital, 5-ethyl-5-acetyl barbituric acid). The mechanism of the hydrogen atom abstraction can be understood in both types of reaction. It is also understandable that the radiation or the argon excitation energy transferred to the molecules can cause release of larger groups like hydroxyl (alloxan), ethyl (barbital) or acetyl (5-methyl-5-acetyl barbituric acid, not described here). But, it is not so well understood how hydrogen atoms of thermal energies can abstract such large groups bound to saturated carbon atoms. The reaction mechanism must be different from that in the conjugated systems[15]. An attempt was made to understand such a reaction in alloxan[13]. However, in these experiments one cannot be quite sure that besides the thermal hydrogen atoms also some of the excited species do not reach the molecules under investigation.

It is interesting that the loss of the substituent (R′ or R″) takes place only in the pharmacologically inactive substances. The only two pharmacologically active barbiturates studied, barbital and cyclobarbital, exhibit the change in the substituted groups. The ease of the change in the substituent might be connected with the pharmacological potency of the substances.

The reaction products found in this investigation in most cases are the same as the products of thermal decomposition of these molecules[9]. Scission of the ring, observed upon heating the specimens, was not found in the present study. Even if it were present the life-time of the formed radicals would be too short to be observed under the present experimental conditions.

The fact that for the same concentration of radicals in dilituric acid generated by the exposure to H atoms and Ar* atoms, that broadening of the lines can be observed only in the former case might suggest that the Ar* radiation rather than energy excitation is transferred to the specimen. Namely, the line broadening is obviously brought about by the spin-spin interactions in certain local regions, presumably surface. Since H atoms penetrate to the crystalline solid approximately 10^{-5} cm[16] the distribution of the radicals generated by H atoms is expected to be to the same depth. The radical distribution in the specimen exposed to Ar* atoms is obviously in a larger volume which is expected if the incident energy is radiation rather than the excitation energy.

The lack of any line broadening in the other compounds studied may be due to the smaller total concentration of radicals in these systems.

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IZVOD

Studij reaktivnih položaja u derivatima barbiturne kiseline pomoću elektronske spinske rezonancije

J. N. Herak i J. J. Herak

Elektronska spinska rezonancija korištena je za identifikaciju slobodnih radikala u derivatima barbiturne kiseline, nastalih reakcijom ovih molekula s termalnim H atomima, ili pobuđenim Ar atomima. Obje vrste reakcije dovode do produkata — radikala sasvim iste strukture, ukazujući da apsorpcija različitih vrsta energije (fizičke i kemijske) dovodi do istih promjena. Kod svih ispitivanih spojeva nađeno je da su aktivna mjesta supstituenti na položaju 5. Opažene su samo dvije vrste reakcije: otcjepljenje supstituenta R′ ili R″ te otcjepljenje protona iz supstituenta. Prva je reakcija prisutna kod farmakološki neaktivnih spojeva. Farmakološki aktivni spojevi (barbital, ciklobarbital) doživljavaju promjenu u supstituentu.

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