Formation of H-Addition Radicals in Adenine Derivatives: Part II

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(Z. Naturforsch. **32 c**, 1–10 [1977]; received October 20, 1976)

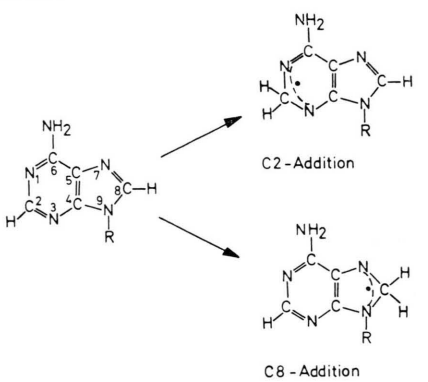
Electron Spin Resonance, Adenine, Radicals, INDO

The formation of H-addition radicals in monocrystals of adenosine, adenosine·HCl, adenine·HCl·$\frac{1}{2}$ H_2O, and adenine·2 HCl by X-irradiation has been studied by using electron spin resonance spectroscopy at 9.5 GHz and at 35 GHz. In all crystals, both H-addition radicals at position C2 and at position C8 were observed. The coupling constants of these two H-addition radicals are different and depend strongly on the protonation state of the adenine base. INDO calculations reproduce well the observed trends of the coupling constants. It is shown that the C2-addition radical is transformed into the C8-addition radical by heat and *vice versa* the C8-addition into the C2-addition by light of $\lambda > 360$ nm.

1. Introduction

In a previous paper (part I), we have studied the formation of H-addition radicals in monocrystals of 9-methyladenine and deoxyadenosine monohydrate [1]. Some preliminary results on single crystals of adenosine·HCl were also presented. We could show that, in deoxyadenosine monohydrate, hydrogen addition occurs at two sites: the carbon C2 of the pyrimidine part of the purine ring and the carbon C8 of the imidazole part of the purine ring. The two H-addition radicals could be separated utilizing their different stability under warming or illumination with light. The ESR parameters of both radicals are different and are well reproduced by INDO calculations.

On the other hand, in single crystals of 9-methyladenine only C8-addition radicals were detected. From preliminary results on adenosine·HCl monocrystals, it appeared that also in these crystals only C8-addition radicals were present. However, INDO calculations have shown later on that, when the purine base is protonated at N1 as in adenosine·HCl crystals, the methylene couplings of both H-addition radicals are roughly identical. In this paper, we present evidence that both H-addition radicals are present not only in adenosine·HCl single crystals but also in adenosine, adenine·HCl·$\frac{1}{2}$ H_2O, and adenine·2 HCl monocrystals. We also show that it is possible to generate and stabilize C2-addition radicals in single crystals of 9-methyladenine and 9-ethyladenine. It will be concluded that the observed relative concentrations of both H-addition radicals in adenine derivatives crystals depend principally on two factors: the preferred site of attack on the adenine base, which in turn depends on the protonation state of the ring, and the differential stability of the produced radicals in the crystalline environment.



2. Experimental

Single crystals of adenosine were grown by slow cooling of aqueous solutions saturated at 60 – 70 °C. The proton bonded to C8 was exchanged for deuterium by warming a D_2O solution of adenosine at 70 – 80 °C for several hours. Since single crystals were grown at a temperature where proton-deuteron exchange occurs at C8, single crystals were obtained either fully protonated or with the C8-proton together with the easily exchangeable protons replaced by deuterons. However, some C8-deuterated

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crystals of less good quality could be grown from H_2O solutions at a lower temperature where there is no back exchange.

Single crystals of adenosine·HCl were grown from 1 N HCl solutions at 4 °C. Crystals obtained were either fully protonated, with the amino and hydroxyl groups deuterated (from DCl solutions), with the amino and hydroxyl groups and the C8-proton deuterated, or with only the proton at the C8 carbon replaced by a deuteron. Crystals of both substances are monoclinic with space group $P2_1$ and two molecules per unit cell [2, 3]. For the ESR measurements, an orthogonal coordinate system a, b, and c^* was chosen.

Single crystals of adenine·HCl·$\frac{1}{2}H_2O$ were grown from 4 N HCl solutions at 4 °C. They are monoclinic with space group $P2/c$ and four molecules per unit cell [4, 5]. An orthogonal coordinate system a, b, and c^* was chosen for the ESR measurements. The adenine·2 HCl monocrystals were grown from 7 N HCl solutions at 4 °C. They are orthorhombic with space group $Pnma$ and four molecules per unit cell [6]. For these two last crystals, the same four types of protonated and deuterated crystals were grown as for adenosine·HCl. The experimental equipment and procedure were as previously [1].

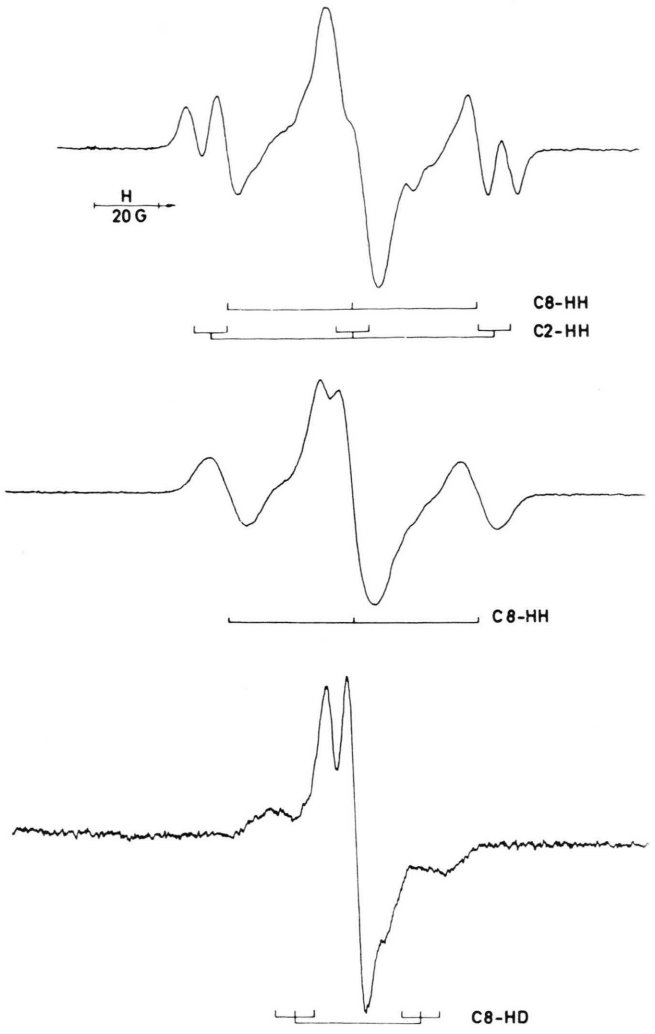


Fig. 1. ESR spectra of single crystals of adenosine X-irradiated at 300 K. For the three spectra the magnetic field is parallel to the purine ring and perpendicular to the C8-H bond. Top spectrum: immediately after X-irradiation. Second spectrum: after subsequent warming to 100–120 °C. Third spectrum: same treatment as for the second spectrum but the crystal was grown from a D_2O solution.

3. Results

3.1. Identification of the radicals

Adenosine

Immediately after X-irradiation at room temperature of single crystals of adenosine, one obtains the top spectrum of Fig. 1. The magnetic field is parallel to the purine rings of the two non-equivalent molecules and perpendicular to the C8-H bond. In order to separate the different radicals present in the spectra taken after X-irradiation at 300 K, we have followed the same procedure as that used with single crystals of deoxyadenosine·H_2O. The second spectrum of Fig. 1 shows the effect of warming for 1 to 2 hours at 100 – 120 °C a crystal of adenosine. Identical treatment on a deuterated crystal yields the third spectrum of Fig. 1. The warming procedure suppresses the extreme lines and thus reduces the overall width of the spectrum. After deuteration of the C8-proton the triplet structure of the second spectrum is replaced by a broad doublet. It follows that the 1:2:1 triplet with 39.0 ± 0.5 G splitting of the second spectrum is due to the C8-addition radical. If, however, the protonated crystal is illuminated with light of $\lambda > 360$ nm at room temperature after X-irradiation, one observes the top spectrum at the left of Fig. 2. This spectrum contains the extreme lines present immediately after X-irradiation and is characterized by a triplet of doublets. Application of the same treatment to a deuterated crystal gives the lower spectrum at the left of Fig. 2: the doublet substructure of the large triplet has disappeared. The same kind of spectrum is obtained with crystals where only the C8-proton

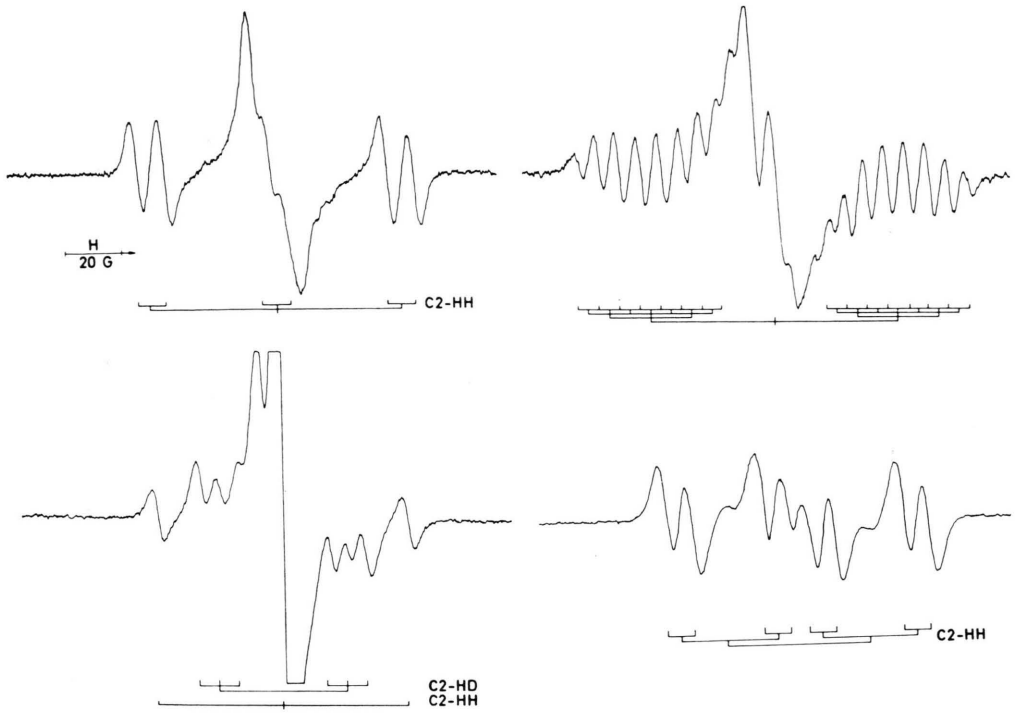


Fig. 2. Left. Top spectrum: ESR spectrum of a single crystal of adenosine X-irradiated and illuminated with light at room temperature. Lower spectrum: *idem* but with a single crystal grown from a D_2O solution. Right. Top spectrum: ESR spectrum of a single crystal of adenosine X-irradiated and illuminated at 300 K showing the nitrogen couplings of the C2-addition radical. The magnetic field is at a skew orientation relative to the normal of the purine ring. Lower spectrum: ESR spectrum of a single crystal of deoxyadenosine monohydrate X-irradiated and illuminated with light at 300 K. Except for the top spectrum at the right, the magnetic field is parallel to the purine ring and perpendicular to the C8-H bond for the spectra.

has been replaced by a deuteron. Therefore, this 1:2:1 triplet with 44.0 G splitting originates from the C2-addition radical and the additional doublet splitting from the proton bonded to C8. When the magnetic field is at a skew orientation relative to the purine ring, hyperfine lines typical of interaction of the unpaired electron with two non-equivalent nitrogen nuclei appear (Fig. 2, top spectrum at the right).

The last spectrum of Fig. 2 is obtained with a single crystal of deoxyadenosine·H_2O after the same treatment. The orientation of the magnetic field is chosen so that it is the same as in the spectra shown on the left: parallel to the purine ring and perpendicular to the C8-H bond. The doublet splitting of 9.5 G of the quartet in deoxyadenosine is identical to that of the triplet in adenosine. The only difference arises from the non-equivalence of the methylene proton coupling constants in deoxyadenosine. We could not observe the equivalent supplementary splitting due to the C2-proton in the C8-addition radical, but only a variation of the width of the lines when the magnetic field sweeps the plane of the purine ring. The coupling constants of both radicals are contained in Table I.

Adenosine·HCl

In Fig. 3 are presented the spectra obtained after X-irradiation at room temperature of single crystals of fully protonated adenosine·HCl and of crystals where the C8-proton has been exchanged for a deuteron (top spectra). The effect of the C8-deuteration is to replace some outer lines by satellites on each side of the large central peak. This indicates that the lines due to the C8-addition radical are present. Upon exposure to light, the same lines as those modified by the C8-deuteration disappear. One is left with a triplet of quartets in the fully protonated crystals and a triplet of doublets in the C8-deuterated crystals. This triplet of 39.0 G splitting is assigned to the C2-addition radical with one of the supplementary couplings coming from the proton bonded to C8.

Table I. Coupling constants (in gauss) of the C2- and C8-addition radicals in the single crystals investigated in this paper. The results for deoxyadenosine·H_2O are taken from reference [1]. The figure in parenthesis indicate the number of equivalent couplings. For the small couplings, the maximum observed value is given; the orientation of the magnetic field is then always roughly perpendicular to the C—H or N—H bond. For the nitrogens, only the values obtained with the magnetic field perpendicular to the ring are given. For the C2-addition radical N′ and N″ are respectively N3 and N1, and for the C8-addition radical N′ and N″ are respectively N7 and N9. The nitrogen couplings for the C8-addition could not be determined because of the large width of its lines and of the unfavourable relative orientation of the two non-equivalent purine rings.

Crystal	Radical	H(C2)	H(C8)	H(N1)	H(N7)	H(N9)	N′	N″
Deoxyadenosine·H_2O	C2-addition	42.0(2)	9.5	—	—	—	20.5	9.5
	C8-addition	—	38.0(2)	—	—	—	27.0	6.5
Adenosine	C2-addition	44.0(2)	10.0	—	—	—	20.5	10.0
	C8-addition	—	39.0	—	—	—	—	—
Adenosine·HCl	C2-addition	39.0(2)	6.5	4.5	—	—	23.0	6.0
	C8-addition	—	40.0(2)	—	—	—	—	—
Adenine·HCl·$\frac{1}{2}$ H_2O	C2-addition	40.0(2)	6.5	5.0	—	—	25.0	—
	C8-addition	6.0	42.0(2)	—	—	—	—	—
Adenine·2 HCl	C2-addition	45.0(2)	—	—	—	—	25.0	—
	C8-addition	—	41.0(2)	—	13.0	7.0	—	—

In crystals of adenosine·DCl, where the protons bonded to nitrogens and oxygens are replaced by deuterons, the other supplementary coupling disappears. In fully deuterated crystals (C8-proton and the easily exchangeable protons replaced by deuterons), one observes the 1:2:1 triplet of the C2-addition radical without any supplementary coupling and also the lines of the C2-D-addition radical. Since in adenosine·HCl the adenosine molecule is protonated at N1, the coupling coming from an easily exchangeable proton is most likely due to the N1-proton. This is in agreement with the orientation dependence of this splitting and with INDO calculations *(vide infra)*.

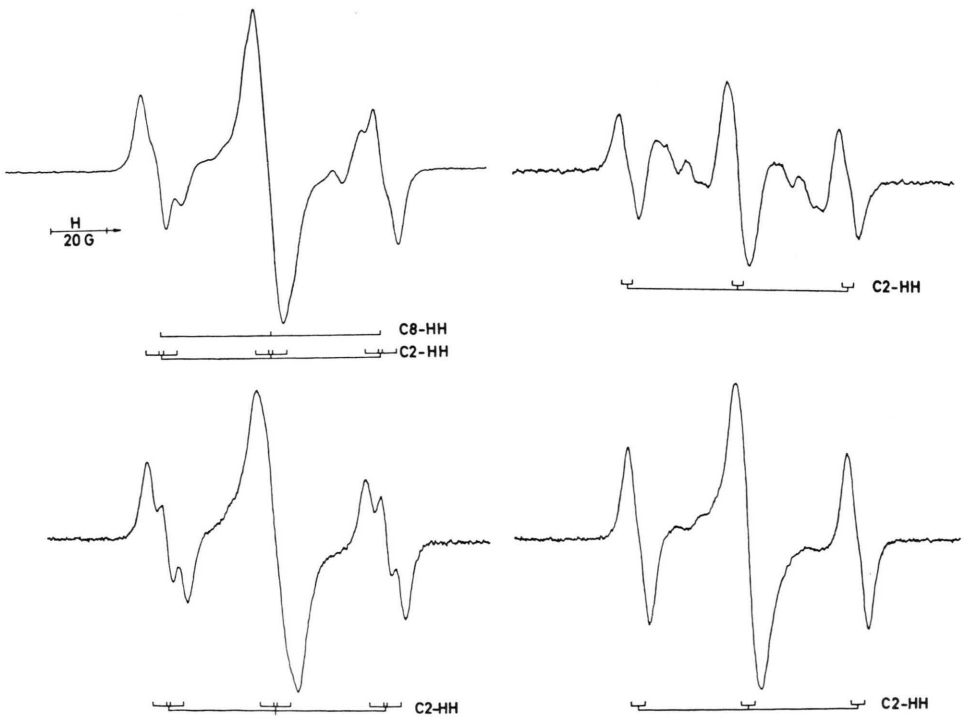


Fig. 3. Left. Top spectrum: ESR spectrum of a single crystal of adenosine·HCl X-irradiated at room temperature. Lower spectrum: after subsequent illumination with light. Right. Same as for the left spectra except that the C8 position was deuterated. The magneic field is for all spectra in the purine plane at 20° from the perpendicular to the C8-H bond.

The coupling constants of both radicals which could be extracted from the spectra are contained in Table I. It should be observed that, in N1-protonated adenine base, both H-addition radicals have similar methylene coupling constants. The coupling due to the C8-proton in the C2-addition radical is also smaller than that in the unprotonated base.

Adenine·HCl·$\frac{1}{2}$ H_2O

Single crystals of adenine·HCl·$\frac{1}{2}$ H_2O have already been studied [7]. It was concluded that the only H-addition radical present was that occuring at C8. This result is at variance with the occurence of both H-addition radicals in the protonated nucleoside. We therefore decided to reexamine single crystals of adenine·HCl·$\frac{1}{2}$ H_2O and to apply our radical separation methods.

The top spectra of Fig. 4 are obtained with crystals of fully protonated and C8-deuterated adenine hydrochloride hemihydrate irradiated at room temperature. The effect of the C8-deuteration is twofold: it removes the extreme lateral lines, which are replaced by broad lines next to the central singlet, and it suppresses the supplementary splitting of the remaining outer lines. A comparison with the case of adenosine·HCl indicates that the extreme lateral lines are due to the C8-addition radical with the outer lines present in the C8-deuterated crystal coming from the C2-addition radical. This assignment of the lines to the two H-addition radicals is corroborated by the lower spectrum at the left of Fig. 4. This spectrum is obtained with a fully protonated adenine·HCl·$\frac{1}{2}$ H_2O crystal which has been illuminated with light after X-irradiation at room temperature. The intensity of the extreme lines due to the C8-addition radical is clearly reduced and the supplementary splitting of the lateral lines belonging to the C2-addition radical is still present.

At other orientations, an irradiated and illuminated crystal reveals the presence of another supplementary coupling in the large 1:2:1 triplet of the C2-addition radical. These two small splittings of the C2-addition radical are roughly identical. Through studies of specifically deuterated crystals, it can be concluded that one splitting arises from the C8-proton and the other one from an easily exchangeable proton. As in the case of adenosine·HCl, we identify this proton with the one bonded to N1, since in adenine·HCl·$\frac{1}{2}$ H_2O crystals the adenine base is also protonated at N1. Another coupling seems to split the lines of the C8-addition radical at some orientations. It is best observed in crystals where all easily exchangeable protons have been replaced by deuterons. The orientation dependence

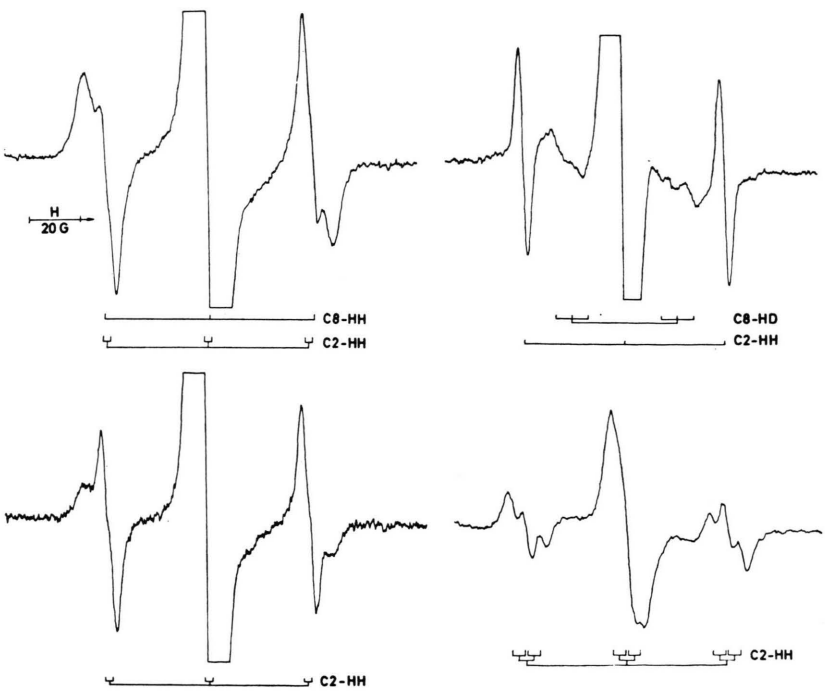


Fig. 4. Left. Top spectrum: ESR spectrum of a single crystal of adenine·1/2 H_2O·HCl after X-irradiation at room temperature. Lower spectrum: after subsequent illumination with light. Right. Top spectrum: ESR spectrum of a C8-deuterated crystal of adenine·1/2 H_2O·HCl after X-irradiation at room temperature. For these three spectra, the magnetic field was parallel to the purine ring at 30° from the C8-H bond. Lower spectrum: same as for the lower spectrum left but with the magnetic field perpendicular to the C8-H bond in the purine plane.

indicates that it should arise from the C2-proton, as it is expected from INDO calculations. In adenosine·HCl crystals, we could not resolve this coupling because of site splitting. The coupling constants of both radicals are presented in Table I.

Another interesting phenomenon was observed in this crystal: upon illumination with light after X-irradiation at 300 K, the central components of the spectrum strongly decrease and the lines of the H-addition radicals increase. This could indicate a probable transformation of the postulated anion [7] underlying the central lines into H-addition radicals.

Adenine·2 HCl

This crystal has also been analysed previously with conclusions concerning the identification of the H-addition radicals similar to those obtained with adenine·HCl·$\frac{1}{2}$ H_2O [7]. This crystal is particularly interesting since the adenine base is doubly protonated, at N1 and at N7.

The spectra shown in Fig. 5 were obtained after irradiation at room temperature of adenine·2 HCl crystals. The spectra of the left were taken with the magnetic field parallel to the *a*-axis (*i. e.* perpendicular to the C8-bond in the molecular plane) and those on the right with the magnetic field parallel to the *c*-axis (*i. e.* parallel to the C8-H bond). Further, the spectra at the top were obtained with fully protonated crystals and those at the bottom with crystals where the C8-proton has been replaced by a deuteron. The large effect of the C8-deuteration on the spectra clearly indicates the presence of C8-addition radicals. The other extreme lines in the lower spectra are then those due to the C2-addition radicals. The hyperfine structure present in the top spectrum on the right is not observed with crystals grown from DCl. A comparison between the fully protonated and the C8-deuterated crystals shows further that this hyperfine structure belongs to the C8-addition radical and not to the C2-addition radical. Since this structure arises from easily exchangeable protons and since the splittings are maximal when the magnetic field is parallel to the C8-H bond (*i. e.* perpendicular to the N7-H and N9-H bonds), we assign these couplings to the protons bonded to N7 and N9. These assignments are in agreement with INDO calculations.

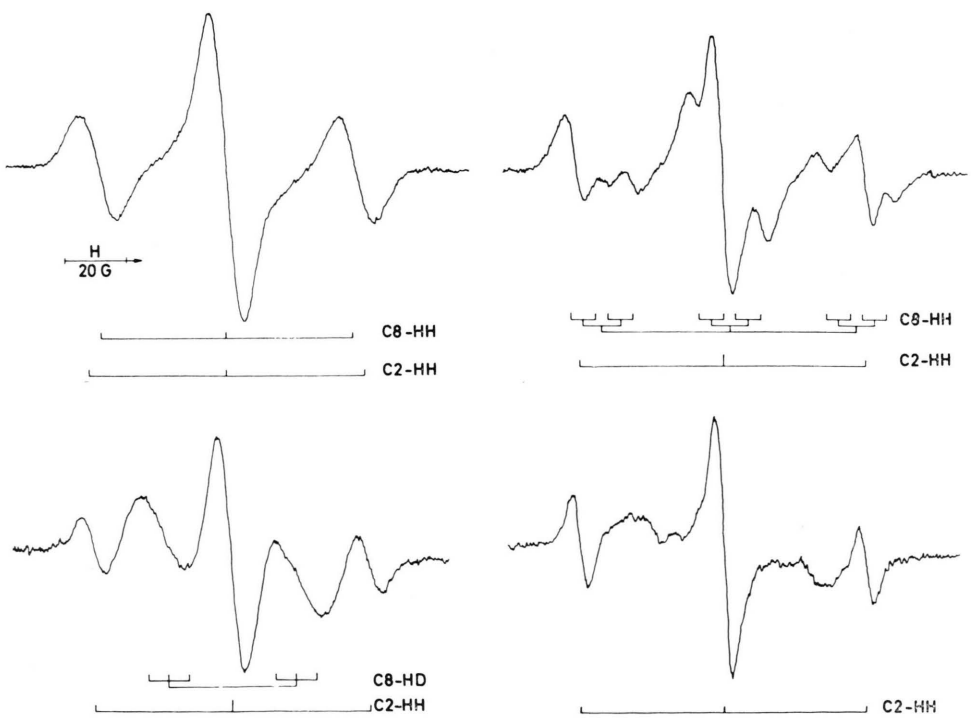


Fig. 5. ESR spectra of single crystals of adenine·2 HCl X-irradiated at room temperature. Left: the magnetic field is oriented parallel to the *a*-axis. Right: the magnetic field is oriented parallel to the *c*-axis. Top: fully protonated crystals. Bottom: C8-deuterated crystals.

Although the radicals could not be separated through annealing or bleaching, careful analysis of the spectra taken at 9.5 GHz and 35 GHz supports the existence of both radicals and the assignment of the supplementary couplings to the C8-addition radical. A complete analysis of all hyperfine tensors was not possible owing to the simultaneous presence of both radicals with large line-widths. The extracted couplings are given in Table I. In the other crystals the concentrations of both H-addition radicals were roughly identical, as far as it could be judged from the spectra at different orientations. However, in the adenine·2 HCl crystals, the concentration of the C8-addition radical is larger than that of the C2-addition radical.

3.2. INDO calculations

From the preceding section, it is evident that the protonation state of the purine base influences greatly the relative magnitudes of the coupling constants of the C2- and C8-addition radicals. These differences were not expected previously. Therefore, in order to complement our specific deuteration studies we have performed several INDO calculations on the purine H-addition radicals.

The procedure was the same as that applied to the imidazole and pyrimidine H-addition radicals [8, 9]. Only the lengths of the bonds between the H-addition site and the rest of the molecule (C-N bonds) were altered and set equal to 1.40 Å. The other bond lengths and bond angles were taken from crystallographic data. The radicals were calculated with the methylene protons at tetrahedral angles to their neighbouring atoms. The results of the calculations given in Table II are those for the adenine molecule. These results are not significantly different from those obtained with 9-CH_3-adenine, which can be considered as a good model substance for the nucleosides. In any case, the results of Table I show that the nucleoside and the base yield identical coupling constants.

A comparison between the coupling constants presented in Tables I and II shows that the magnitudes of the calculated coupling constants of the methylene protons and of the nitrogens are too large. However, in this class of H-addition radicals, all relative values associated with the protonation state of the purine base are well reproduced by the calculations. First, in the free base, the significant difference in the methylene proton coupling constants of both H-addition radicals and in the relative magnitude of both nitrogen couplings is well reproduced. Also, the supplementary coupling of the C8-proton for the C2-addition radical is larger than that of the C2-proton for the C8-addition radical. Secondly, the coupling due to the C8-proton in the C2-addition radical is larger in the free base than in the N1-protonated base. Also, for the N1-protonated base, the absence of coupling to the N1-proton in the C8-addition radical and its occurence in the C2-addition radical is reproduced. Thirdly, in the N1, N7-protonated base, the C2-addition radical presents no supplementary coupling, while the C8-addition radical has hyperfine couplings with the protons bonded at N7 and at N9, the one bonded to N7 giving the largest.

It should also be noted that the relative magnitude of the methylene proton couplings of both radicals in the N1-protonated base and in the N1, N7-protonated base are recovered. Thus, in the N1-protonated base, they are roughly identical and, in the N1, N7-protonated base, they are larger for the C2-

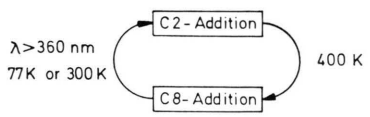
Table II. Coupling constants (in gauss) of the different H-addition radicals of the adenine base in various protonation states as calculated by the INDO MO method. For the protons the isotropic part is given and for the nitrogens the value which should be observed with the magnetic field perpendicular to the ring. The isotropic values of the small protons are not directly comparable to the coupling constants presented in Table I. However, the greater the isotropic part, the greater the maximum observed coupling.

Protonation state	Radical	H(C2)	H(C8)	H(N1)	H(N7)	H(N9)	N′	N″
Free base	C2-addition	67.8(2)	−4.0	—	—	−0.2	25.6	18.2
	C8-addition	−2.7	47.1(2)	—	—	−1.2	31.2	1.5
N1-protonated	C2-addition	49.4(2)	−2.1	−2.2	—	−1.3	29.2	3.5
	C8-addition	−2.8	49.9(2)	0.8	—	−0.8	29.9	3.0
N1- and N7-protonated	C2-addition	54.2(2)	0.4	−2.9	−0.6	−1.4	29.3	5.0
	C8-addition	−0.9	51.1(2)	0.6	−11.0	−3.9	24.6	7.5
N7-protonated	C2-addition	73.2(2)	−1.1	—	1.1	−0.9	25.6	21.7
	C8-addition	−2.2	43.8(2)	—	−9.9	−3.4	22.2	6.2

addition than for the C8-addition radical. The relative trends in the nitrogen couplings are followed, the agreement being less good for the C2-addition radical of the free base. This radical is most sensitive to molecular changes as can be seen from the results obtained with the N7-protonated base. We are not sure that we have calculated the C2-addition radical of the free base in the correct configuration or tautomeric from *(vide infra)*.

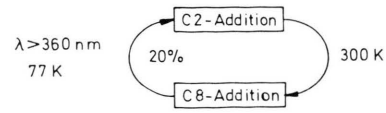
3.3. Light- and heat-induced radical transformations

The spectra of an adenosine crystal X-irradiated at room temperature and warmed for 30 min at 100 °C present only the lines of the C8-addition radical. When a crystal so treated is illuminated with light of $\lambda > 360$ nm, the lines of the C8-addition radical completely disappear and those of the C2-addition radical appear. After a further annealing of the crystal at 100 °C, the lines of the C2-addition radical decrease and those of the C8-addition reappear. It is thus possible to transform the C2-addition radical into the C8-addition radical by heat and the C8-addition radical into the C2-addition radical by light. These reactions are fully reversible and the process may be repeated several times without loss of the total radical concentration. These reactions occur also in deoxyadenosine·H_2O crystals. In deoxyadenosine·H_2O crystals, the light-transformation of C8-addition radical into C2-addition radical is best performed at 77 K and not at 300 K. Therefore, in crystals of adenine nucleosides containing the neutral base, one has the following transformation cycle:



It has been observed in C8-deuterated crystals that only hydrogen atoms are displaced by heat or light and not deuterium atoms.

Since it was not clear why these transformations should not occur in crystals of 9-CH_3-adenine, we reexamined such crystals and found that the light-transformation can only be observed if the illumination takes place at 77 K because the C2-addition radical is not stable at room temperature. Further, even at 77 K, only 20% of C8-addition radicals could be transformed into C2-addition radicals. An exactly similar behaviour happens in crystals of 9-ethyladenine. For crystals containing the adenine free base alkylated at the nitrogen N9, the following transformation scheme is valid:



In crystals containing the protonated base of the adenine molecule, the C2-addition is always stable against heat. However, in N1-protonated adenine derivative crystals, the C8-addition radical can be bleached by visible light with only a small fraction transforming into C2-addition radical. The adenine·2 HCl crystals have the peculiarity of presenting none of these transformations: neither the C2-addition radical can transform by heat nor the C8-addition radical can be bleached by visible light.

4. Discussion

4.1. Crystalline environment and radical stability

The relative concentrations of both H-addition radicals are very dependent on the crystalline environment. In adenine derivative crystals dominated by van-der-Waals forces, like 9-CH_3-adenine, only C8-addition radicals are detected. On the other hand, in crystals containing small polar molecules or in crystals of adenine nucleoside, which are characterized by an extensive hydrogen bonding scheme and a more ionic environment, both H-addition radicals occur. Further, in adenine·2 HCl crystals, the concentration of C8-addition radicals is larger than that of C2-addition radicals. Also, in the protonated base, it is not possible to transform the C2-addition radical by heat. However, C2-addition radicals can be produced from C8-addition radicals by illuminating with light at 77 K single crystals of adenine free base alkylated at N9. Upon storage at room temperature, the C2-addition converts back into C8-addition radicals.

All these observations can be rationalized by assuming that the C2-addition radical needs a specific environment to be stabilized. From the trends in the relative H-addition radical concentrations, it can be said that an ionic environment preferably stabilizes the C2-addition radicals. It is, however, not straightforward to find out the structural reasons of this feature. We thought of an additional protonation of

the C2-addition radical, for example at N7. From Table II, it is clear that this is not the case. We have also calculated the C2-addition radical with a skew amino group. This does not change significantly the couplings. There is, nevertheless, a molecular property of the C2-addition radical which could be responsible for its stability in an ionic environment: its calculated dipole moment is larger than that of the C8-addition radical (2.7 D instead of 1.7 D). This slight difference could account for the instability of the C2-addition radical in the environment of the 9-CH_3-adenine crystals which can be regarded as hydrophobic. The same would hold for the increased stability of the C2-addition radical at room temperature in the hydrogen-bonded nucleoside crystals and for its stability at 100 °C in crystals containing a protonated form of the adenine base.

4.2. Sites of attack

Our conclusions in the previous paper [1] concerning the sites of attack in adenine derivatives have to be corrected as follows in the light of the present results. Independent of the protonation state, H-addition radicals are formed at position C8 of the imidazole part and at position C2 of the pyrimidine part of the adenine base. The observed relative concentrations of both radicals after irradiation at room temperature also depend on the crystalline environment. If C2-addition radicals are produced by radiation initially they can convert back into C8-addition radicals in a nonpolar environment.

H-addition radicals may be the result of either direct transfer of atomic hydrogen or of primary displacement of an electron followed by a neutralizing proton transfer. We could not distinguish between these two reaction mechanisms in the cases of the free base and the N1-protonated adenine derivatives. Hückel molecular orbital calculations indicate that attack by free radical occurs slightly preferentially on C8 in the free adenine base [10]. This could also contribute to the exclusive presence of C8-addition radical in 9-methyladenine. The distinction must be very weak, since in the adenine nucleosides attack occurs at C2 and at C8.

The distinction between C2 and C8 is even less clear in case of protonation of the electron adducts of the free base or the N1-protonated adenine molecule. The preference for C8 is, however, increased over that for C2 in case of protonation of the electron adduct of the doubly protonated base. This is revealed by the predominant concentration of C8-addition radicals in adenine·2 HCl crystals. In such crystals, the anion radical has been identified by Box and Budzinsky [11]. The main spin and electron density of this radical is localized on C8, which should make that carbon a good site of attack for protons. It is not possible to say that the C2-addition radicals are formed through direct hydrogen addition or that the C8-addition radicals are formed exclusively through potonation of the electron adduct.

5. Conclusions

In the previous ESR investigations concerning the formation of H-addition radicals in adenine derivatives, some uncertainties about the site of addition remained [12–15]. For the first time, Schmidt and Borg [16] indicated the presence of both types of H-addition radicals in most polycrystalline samples they studied. We conclude also that both radicals are produced by X-irradiation but, since the C2-addition radical needs a specific environment to be stabilized, its concentration is strongly dependent on the crystal studied. We further consider that an initial preference for C8 is only marked when the H-addition radical is produced through protonation of the electron adduct of the doubly protonated base.

From the ESR point of view, these H-addition radicals are interesting because of the drastic changes in their couplings provoked by protonation. INDO calculations were particularly useful to support and rationalize the specific deuteration studies.

The observed transformation of the C8-addition radical into the C2-addition radical upon irradiation with visible light greatly facilitated the identification of the two radicals. Conversion of hydrogen addition radicals by illumination and heat was also observed in pyrimidine single crystals [9].

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