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A Quantum-Mechanical Study of the Interaction of Glyoxal with Guanine

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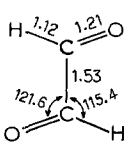
A quantum molecular study by the SCF *ab initio* method of the interaction of glyoxal with guanine provides for the formation of a stable covalent adduct in which the glyoxal fragment forms a complementary cyclic ring attached to the imino N_1 and amino N_2 atoms of guanine with the concomitant migration of the N-bonded H atoms to the oxygens of glyoxal. The reaction should proceed in two steps. The most plausible mechanism involves as the first step the interaction of a carbonyl group of glyoxal with the amino group of guanine followed by a similar interaction at the imino group of guanine, rather than the reverse order of interactions. The respective energy barriers are 49.7 and 63.9 kcal/mole. The intermediate product is also more stable when the adduct occurs first at N_2: 30.7 kcal/mole versus 17.9 kcal/mole for the adduct at N_1.

Key words: Glyoxal, interaction of ~ with guanine

1. Introduction

Glyoxal (I) and some of its derivatives are known to react with different types of nucleic acids bringing about important biological consequences. They have been shown to be able to inactivate the RNA of tobacco mosaic virus [1] or the yeast phenylalanine transfer ribonucleic acid [2] and to denature DNA [3, 4]. They also exert a cancerostatic activity which has been indicated by Albert Szent-Gyorgyi *et al.* in 1967 [5] and stressed by him in recent papers [6, 7].



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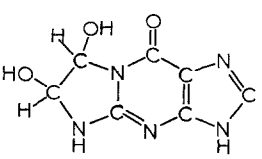

II

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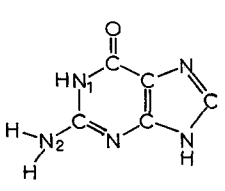
The biological consequences of the interaction of glyoxal with nucleic acids seem to result from its interaction with the nucleic acid bases and particularly with the guanine residue: while the products of its interaction with adenine and cytosine are unstable, the guanine adduct is stable. Its structure has been shown by Shapiro *et al.* [8–10] to consist of a cyclo-addition of the carbonyl groups of glyoxal to the endocyclic imino-N_1 and exocyclic amino-N_2 nitrogen atoms of guanine, yielding II. The hydroxyl groups of the new pentacycle are gauche with respect to each other [9]. The reaction is faster at neutral than at acidic pH. The adduct is stable for 48 hours at room temperature up to pH 9, but reverts quickly to guanine at pH 11. Periodate degradation leads to N_2-formyl guanine not to N_1-formyl- or N_1, N_2-diformyl guanine.

Complementary information which may be useful for the elucidation of the nature of the reaction is provided by a recent study of McGhee and von Hippel [11] on the interaction of formaldehyde with guanine and other DNA bases to form N-hydroxymethyl derivatives. These authors find that both the N_1 endocyclic imino group and the amino group can react with this reagent. The reaction with the exocyclic amine nitrogen is rather slow and pH independent, while the reaction with the imino group is quite fast and subject to specific base catalysis. Equilibrium constants are such that at neutral pH the N_2-adduct would be the dominant product. An activation enthalpy of 14.7 kcal/mole was found for the overall reaction.

On the other hand from the theoretical point of view, a recent investigation by the CNDO/2 method on the interaction of glyoxal with the DNA bases was used by its author to propose the possibility of a competition in hydrogen bonding between the formation of complementary DNA bases pairs and the pairing of the bases with glyoxal [11]. This suggestion not only conflicts with the experimental data on the nature of the reaction products but implies rather improbable N···H–C or O···H–C hydrogen bonds. The computed interaction energies for such glyoxal-bases hydrogen bonded complexes are moreover much smaller than those computed similarly for the complementary base-pairs. These factors disfavor the possibility of the postulated competitivity.

In the present paper we propose to investigate theoretically in more detail the possible mechanism of the glyoxal-guanine interaction.



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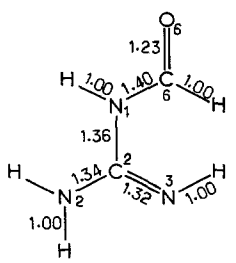


IV

2. The Method

The computations have been carried out by the SCF MO *ab initio* method using the program Gaussian 70 with the standard STO-3G basis set [12]. To reduce computational costs, we have represented guanine by the realistic model IV which gives a satisfactory representation of the part of the molecule involved in the reaction with glyoxal. To mimic the rigidity of the cyclic structure of guanine, we have kept IV frozen at the experimental geometry of that base [13]. Glyoxal was also taken initially in its experimental, *trans* geometry [14]. I and IV indicate these geometries.

We have not attempted a detailed study of the potential surface for the reaction, a hopeless task for such a complicated system. By studying selected points on the surface, we hope to have obtained a satisfactory qualitative description of the main features of the reactions, both at N_1 and at N_2, of their similarities and differences.

In our choice of these selected points, we have been guided by the existing knowledge, experimental and theoretical, on the gross features of the mechanism of related reactions involving the addition of a carbonyl group on aliphatic amines [15]. Such reactions are considered to involve a nucleophilic attack of the lone pair of the nitrogen atom upon the carbon atom of the carbonyl group, the nitrogen approaching in a direction perpendicular to the plane of the carbonyl group. Either the formation of the C–N bond, or the proton transfer from N to O may be rate limiting.

Bürgi *et al.* [16, 17] have studied the geometrical aspects of this mechanism at the molecular level. They used the analysis of suitable crystal structures to delineate the changes in the geometry of the carbonyl group as a function of the distance of the nucleophilic nitrogen atom to the carbonyl carbon atom. The shorter the N···C distance the more the carbonyl group is pyramidalized at the carbon atom towards an extreme tetrahedral structure. They expressed their results in terms of empirical equations relating the changes in these geometrical parameters. The out-of-plane displacement (Δ) of the electrophilic C atom out of the plane defined by its three bonded atoms (the carbonyl O and the two R, R′ atoms attached to C) towards the nucleophilic centre, follows a logarithmic relationship to the C–N bond distance d_1:

$$d_1 = -1.701 \log_{10} \Delta + 0.867 \text{ Å}$$

which can also be reformulated as

$$d_1 = -1.701 \log_{10} n + 1.479 \text{ Å}$$

where $n = \Delta/\Delta_{max}$ and Δ_{max} is the out of plane displacement calculated for $d_1 =$ 1.479 Å, the standard C–N bond length.

At the same time the C–O bond length increases, as d_1 decreases, roughly according to the relationship

$$d_2 = 0.71 \log_{10} (1 - n) + 1.426 \text{ Å}.$$

Molecular orbital computations by Bürgi *et al.* [18] on the model reaction $H^- + H_2C{=}O \rightarrow CH_3O^-$ were found to be in general agreement with this formulation.

We have thus used it as a guide in the geometry variations at the points of attack in the search of the reaction path.

3. Results and Their Discussion

Since the overall reaction consists of a double nucleophilic addition, at the N_2 amino and N_1 imino nitrogens, the first question that may be raised in connection with its mechanism is whether the reaction is a concerted one or a two-step one. In the latter case the second obvious question arises at which site does it start. During preliminary test calculations, we found a strong energy requirement for an approach in which the C···N axis makes an angle of 90° with the molecular plane of guanine; e.g. at a C···N distance of 2.0 Å, increasing this angle to 125° raises the energy by 17 kcal/mole. Inspection of the geometries shows that it is impossible to come close to a configuration of approach in which the glyoxal molecule has simultaneously one of its carbon atoms above N_1 and the other above N_2. Thus a concerted attack does not seem probable and we have therefore concentrated our attention on the two-step reactions. The sequence of events could then be either the addition of one carbonyl of glyoxal at the N_2 amino group of guanine, followed by cyclization through the addition of the other carbonyl to the N_1 imino group, or conversely the first addition at the N_1 imino group, then formation of the cycle by the closure of the ring at the N_2 amino group. We shall study these two possibilities in succession.

3.1. The Reaction Path through the Primary Attack on the Amino Group Followed by Cyclisation on the Imino Nitrogen

We shall investigate first the first half of the reaction, namely the nucleophilic addition of a carbonyl group of glyoxal to the N_2 exocyclic amino nitrogen of guanine. The geometrical changes considered are indicated in the upper part of Table 1.

The distances in the cycle of guanine, the C–C and C–H distances and the geometry of the non-reacting carbonyl group are kept frozen. On the other hand changes occur in the C···N distance, R_{CN}, the geometry of the attacking C=O group, Θ_{CO}, that of the $-NH_2$ group, Θ_{NH_2}, and the position of the H atom transferred during the reaction, H_{tr}. These are thus our four main variable parameters. For the carbonyl group, going from planar to tetrahedral geometry means bending the CH, C–O and C–C bonds by an angle Θ_{CO} of 19°47 away from the C···N axis (e.g. $\widehat{NCO}$ goes from 90° to 109°47). The change in the C–O bond length is coupled with this angular change in accordance with the empirical relationship of Bürgi indicated above. For the amino group, Θ_{NH_2} = planar means a guanine C_2–N_2 distance of 1.34 Å and planar geometry around N. Θ_{NH_2} = tetrahedral means a guanine C_2–N_2 distance of 1.46 Å and tetrahedral angles around N. This distance of 1.46 Å was found by energy optimization of the product of the first half of the

Table 1. Geometries and energies for the reaction between guanine and glyoxal (starting at the amino N_2, terminated at the imino N_1)

Geometry R_{CN}(Å)	Θ_{CO}	Θ_{NH}	H_{tr}	Energy ΔE (kcal/mole)
First half reaction				
∞	0°	pl[a]	on N	0.0
2.5	0°	pl	on N	7.9
2.5	9.85°	pl	on N	14.6
2.0	0°	pl	on N	51.4
2.0	9.85°	pl	on N	43.4
2.0	15.0°	pl	on N	54.5
2.0	0°	td[b]	on N	27.0
2.0	9.85°	td	on N	22.3
1.75	9.85°	td	on N	44.0
1.75	transition state: H between N and O			49.7
2.0	td[c]	td	on O[d]	28.3
1.75	td	td	on O	−11.0
1.5	td	td	on O	−30.7
Second half reaction				
4.50	0°	pl	on N	−30.7
2.14	0°	−20°[e]	on N	25.1
2.14	9.85°	−20°	on N	21.0
1.91	9.85°	−20°	on N	50.5
1.76	0°	−50°	on N	64.0
1.76	transition state: H between N and O			66.3
1.91	td		on O	−1.5
1.76	td		on O	−7.9
1.50	td		on O	−55.8

[a] Planar, $R_{C-N}=1.34$ Å.
[b] Tetrahedral, $R_{C-N}=1.46$ Å.
[c] $\Theta=0°$, $R_{C-O}=1.208$ Å; $\Theta=9.85°$, $R_{C-O}=1.257$ Å; $\Theta=15°$, $R_{C-O}=1.319$ Å; $\Theta=19.47°=$td, $R_{C-O}=1.428$ Å.
[d] $R_{O-H}=0.96$ Å, COH = 109°47.
[e] Angle of N–H bond with the molecular plane of guanine, pl = 0°.

reaction. For all geometries where we have taken NH_2 tetrahedral, in this first half reaction, we have verified that $R(C_2-N_2)=1.46$ Å was always energywise better than $R(C_2-N_2)=1.34$ Å by 6 to 9 kcal/mole. For the transition state model, we also verified that an intermediate value of $R(C_2-N_2)=1.40$ Å led to a higher energy.

At a distance R_{CN} of 2.5 Å, the potential surface for the interaction between the undistorted fragments is repulsive by 7.9 kcal/mole.

Distortion of the carbonyl group by $\Theta_{C=O}=9.85°$ does not improve the energy. If we bring the two undistorted fragments closer to one another, at $R_{CN}=2.0$ Å, the energy raises considerably (+51.4 kcal/mole). Distortion of the fragments is now, however, effective in reducing the repulsion. Distortion of the carbonyl group by an angle $\Theta_{C=O}=9.85°$ gives an energy of +43.4 kcal/mole. Further distortion,

$\Theta_{CO}=15°$, is, however, unfavorable and raises the energy to 54.6 kcal/mole. On the other hand allowing a tetrahedral geometry to the guanine amino group is much more effective in reducing the repulsion. Coupling these two distortions ($\Theta_{CO}=9.85°$, $\Theta_{NH}=$ td) leads to an arrangement only 22.3 kcal/mole above the separated fragments for $R_{CN}=2$ Å. A further decrease of R_{CN} to 1.75 Å raises the energy to 44.0 kcal/mole.

Thus, bringing together the glyoxal and guanine molecules without transferring a proton from N to O raises the energy. In a somewhat similar study on the simpler system NH_3+H_2CO, Scheiner *et al.* [19] found similarly a repulsion of 10.8 kcal/mole at $R_{CN}=2.12$ Å and of 26.8 kcal/mole at $R_{CN}=1.85$ Å.

We may also look at the reaction by starting from the product V and stretching the C–N bond. In the product V, we have reached $R_{CN}=1.50$ Å, a tetrahedral geometry around N and C ($\Theta_{C=O}=\Theta_{NH}=$ td) and the hydrogen atom is on O. The detailed conformation minimizes the occurrence of undesirable close atomic contacts between the unreacted carbonyl group of glyoxal and the guanine cycle. The bonds to the glyoxal carbon atom and those to the guanine nitrogen atom are in a staggered conformation, and the $C_2N_2C_1O_1$ torsion angle is 60°. The torsion angle $N_2C_1C_3O_3$ between the CN bond and the unreacted carbonyl group of glyoxal is 90°. The $O_1C_1C_3O_3$ framework of glyoxal is still nearly *trans* (torsional angle $O_1C_1C_3O_3=160°$). The computations indicate a stabilization of -30.7 kcal/mole with respect to the separated original fragments. If we increase R_{CN} to 2.0 Å, all other parameters remaining unchanged, the energy rises very fast: -11.0 kcal/mole at 1.75 Å, $+28.3$ kcal/mole at 2.0 Å. At this stage ($R_{CN}=2.0$ Å), changing the geometry around CO to $\Theta_{CO}=9.85°$, its optimal value when H is on N at the same R_{CN}, raises the energy further by 13.0 kcal/mole. Thus the carbonyl group achieves a fully tetrahedral geometry only when the H atom is transferred from N to O, while the amino group achieves tetrahedral geometry as soon as $R_{CN}=2.0$ Å.

The most delicate step to describe is the proton jump from N_2 of guanine to the O of glyoxal. The optimization of the energy for the transition state being a difficult task in our system, we took into account information available in the field from two studies on comparable although smaller systems studied in detail recently by two groups of authors. The systems are

$$OH^- + H_2NCOH \longrightarrow HCOO^- + NH_3$$

studied by Alagona *et al.* [20] and

$$CO_2 + OH_2 \longrightarrow H_2CO_3$$

studied by Jönsson *et al.* [21]. In a first attempt, at $R_{CN}=2.0$ Å, the hydrogen atom was placed at 1.45 Å from N and 1.67 Å from O; the carbonyl group was half-pyramidal and the amino group was pyramidal. Valence angles were kept normal. The energy obtained was very high: 94 kcal/mole.

When in the same configuration the C_1O_1H and guanine N_2H valence angles were distorted in a manner similar to that found by Alagona *et al.* [20] implying among others the hydrogen to be placed midpoint between N and O ($R_{C_2N_2}=2.0$ Å,

$R_{O_1H} = R_{N_2H} = 1.38$ Å, $\widehat{N_2C_1O_1} = 90°$, $\widehat{C_1O_1H} = 85°$, $\widehat{C_2N_2C_1} = 125°$) the energy dropped to 81 kcal/mole, 59 kcal/mole above the value of 22.3 kcal/mole corresponding to the presence of H on N. This barrier is much higher than that found by Alagona *et al.* in their reaction (35 kcal/mole) probably because of its ionic nature.

Using a geometry similar to that of Jönsson *et al.* [21], ($R_{O_1H} = 1.28$ Å, $R_{N_2H} = 1.23$ Å, $\widehat{N_2C_1O_1} = 90°$, $\widehat{C_1O_1H} = 79°$, $\widehat{C_2N_2C_1} = 132°$) with $R_{C_2N_2} = 1.75$ Å leads to a more satisfactory energy value of 49.7 kcal/mole for the midpoint of the proton jump. In fact this value is even lower than that found by Jönsson *et al.* for the $H_2O + CO_2$ reaction, although these authors used a much more extended basis set than we could do and included a part of the correlation energy through a CI treatment. Apparently a rather short C–N distance (1.75 Å) and distorted values of the valence angles at the atoms involved in the reaction are necessary to facilitate the proton jump and to obtain a reasonable energy barrier.

We may now proceed to the study of the second half of the reaction which involves the closure of the ring by a nucleophilic addition of the N_1 imino nitrogen atom to the remaining carbonyl group of glyoxal. The corresponding data of our computations are presented in the lower part of Table 1.

In the final product, the newly formed five-membered ring, having a saturated N_2–C_1–C_3 fragment, is non-planar. The optimum geometry, at -55.8 kcal/mole, is found to have only one atom out of the plane, the C atom bound to N_2. An alternative form where one carbon is below and the other above the plane, is less stable (-34.7 kcal/mole). In this less stable form, the C_3–N_1 bond is not coplanar to the hexacycle of guanine.

For forming the C–N bond, we start from the product of the first half reaction at large distance: $R_{CN} = 4.50$ Å, $E = -30.7$ kcal/mole. Reducing R_{CN} to 2.14 Å and lowering the hydrogen bound to N_1 below the plane of the ring ($\Theta_{NH_2} = $ td), raises the energy to $+25.1$ kcal/mole. Bending the carbonyl group to $\Theta_{CO} = 9.85°$ has only a small effect ($E = 21.0$ kcal/mole), and a further decrease of R_{CN} to 1.91 Å raises the energy to 50.5 kcal/mole while at $R_{CN} = 1.76$ Å, it goes up to 64 kcal/mole.

Again one may start the interaction from the final product, with H at O, COH pyramidal and $R_{CN} = 1.5$ Å, to which corresponds $E = -55.8$ kcal/mole. As we stretch the C–N bond while keeping $\widehat{COH}$ pyramidal, as was found optimal in the first half reaction, the energy raises to -7.9 kcal/mole ($R_{CN} = 1.75$ Å) then to -1.5 kcal/mole ($R_{CN} = 1.91$ Å). Our best model for a transition state has an energy of $+66.3$ kcal/mole. It is again modelled on that of Jönsson *et al.* [21], with $R_{CN} = 1.76$ Å and the proton midpoint between O and N.

This leads to an energy barrier of 97 kcal/mole, thus higher than the barrier of 49.7 kcal/mole found for the reaction at the N_2 amino group. Because it was seen earlier that an important factor contributing to the lowering of the barrier for the addition at N_2 is the possibility to stretch the C_2–N_2 bond of guanine, it may be supposed that this difference is to some extent due to the rigidity adopted for the

guanine system in the vicinity of N_1. Complementary information on this aspect of the problem will be found in the second part of this section.

In summary we may say that in the study of the first possible pathway for the reaction of glyoxal with guanine (addition first at N_2, then at N_1) we find that the reaction occurs in two steps with a stable intermediate and that its final product is remarkably stable. In the newly formed pentacycle, an envelope geometry is strongly preferred, in which only one atom, the C_1 bound to N_2, is out of the molecular plane. In both half-reactions, the approach of the reactants contributes to the energy barrier, and after the proton transfer, the energy decreases continuously as the reaction proceeds to the product. The lowest energy barrier is obtained for the proton transfer at a C···N distance of about 1.75 Å. The carbonyl group remains slightly pyramidal when the proton is on N, and becomes totally tetrahedral only when the transfer has occurred. The NH_2 group assumes very early in the reaction a tetrahedral geometry in which the guanine C_2–N_2 distance increases strongly.

3.2. The Reaction Path through the Primary Attack on the Imino Nitrogen Followed by Cyclisation at the Amino Group

We may now describe our results for the second possible reaction pathway: attack of glyoxal on the N_1-imino group, followed by cyclisation at the N_2-amino group. Besides the attempt to establish which of the two reaction pathways is preferred and which intermediate product (at N_1 or at N_2) is more stable, the second goal of this study is to explore the reaction between the carbonyl and intracyclic imino group in greater detail, free from the steric constraints imposed by the formation of the cycle.

The geometries and energies corresponding to this second possible pathway are reported in Table 2. The distortion of the intracyclic angle $\Theta_{C_2N_1C_6}$ is accompanied by an elongation of the C_2–N_1 and C_6–N_1 bonds as follows: at $\Theta = 125.9°$, $R_{C_2N_1} = 1.36$ Å, $R_{C_6N_1} = 1.39$ Å (as in isolated guanine); at $\Theta = 118°$, $R_{C_2N_1} = 1.42$ Å, $R_{C_6N_1} = 1.44$ Å; and at $\Theta = 114.5°$, $R_{C_2N_1} = 1.45$ Å and $R_{C_6N_1} = 1.47$ Å. The deformations are made in such a way that only the N_1 atom is allowed to move, in the molecular plane, in order to mimic the rigidity of the rest of the guanine molecule.

At large distance ($R_{CN} = 2.5$ Å), the best geometry of approach is found to be the one in which the C atom of glyoxal is above N_1 and the bonds are in a staggered conformation. The energy raises to 14.9 kcal/mole. Reducing R_{CN} to 2.0 Å raises the energy considerably. In order to avoid too high an energy, it is necessary to distort the carbonyl group of glyoxal to a half-pyramidal geometry (but no more), and to tilt the CN and NH bonds while keeping a nearly tetrahedral CNH angle. This leads us to a configuration at 49.6 kcal/mole. To further lower the energy one must allow for a deformation of the guanine cycle at N_1. We have verified that in isolated guanine, the geometry corresponding to $\Theta_{C_2N_1C_6} = 125.9°$ is the lowest. The computations show that in the interacting system at $R_{CN} = 2.0$ Å, the nitrogen atom assumes a quite different geometry, closer to tetrahedral, with the C_2–N_1

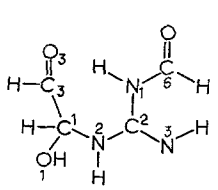
Table 2. Geometries and energies for the addition of glyoxal to guanine at N_1 [a]

Geometry R_{CN_1}	Θ_{CN_1}	Θ_{C-O}	Θ_{N_1H}	$\Theta_{C_2N_1C_6}$	Energy ΔE (kcal/mole)
∞	90°	0°	0°	125.9°	0.0
2.5	90°	0°	0°	125.9°	14.9
2.0	90°	9.85°	0°	125.9°	61.6
2.0	80°	9.85°	20°	125.9°	53.7
2.0	50°	9.85°	60°	125.9°	56.9
2.0	60°	9.85°	50°	125.9°	53.1
2.0	50°	9.85°	50°	125.9°	51.4
2.0	60°	9.85°	40°	125.9°	49.6
2.0	60°	15.0°	40°	125.9°	57.2
2.0	60°	9.85°	40°	118.0°	38.6
2.0	60°	9.85°	40°	114.5°	38.7
2.0	60°	9.85°	50°	118.0°	40.0
1.75	60°	9.85°	40°	118.0°	73.3
1.75	transition state: H between N and O				63.9
1.75	0°	td	H on O	118°	1.1
1.48	0°	td	H on O	118°	−17.9
1.48	20°	td	H on O	118°	−16.8
1.48	0°	td	H on O	125.9°	−7.9

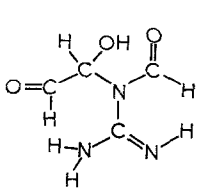
[a] Θ_{C-N}: angle between the C–N bond being formed and the plane of the guanine ring Θ_{C-O}: as in Table 1; Θ_{NH}: angle between the N_1–H_1 bond and the plane of the guanine ring. $\Theta_{C_2N_1C_6}$: intracyclic angle at N_1; angular deformation coupled with bond lengths changes (see text).

and C_6–N_1 bonds intermediate in length between single and double bonds. The energy of the system is then +38.6 kcal/mole. Further deformation of the cycle or modifications of Θ_{NH} or Θ_{CN} do not lower the energy.

If we decrease R_{CN} to 1.75 Å without modifying the other parameters, the energy raises to +73.3 kcal/mole. This is, however, too rigid a modification and a transition state geometry at $R_{CN} = 1.75$ Å with H between N_1 and O, and the angles and distances taken from Jönsson *et al.* [21] leads to an energy of only +63.9 kcal/mole. Other attempts to find geometries with $R_{CN} = 1.75$ Å and H on N_1 lead to even higher energies, which might indicate that the proton jump is coupled with the formation of the CN bond. Keeping R_{CN} at 1.75 Å, when the proton is transferred on O, the COH group assuming tetrahedral angles and the C–N bond being in the guanine plane, the energy drops to 1.1 kcal/mole. A further decrease of R_{CN} to 1.48 Å leads to a final state for the first half-reaction at N_1, VI, at −17.9 kcal/mole.



V



VI

Attempts to move the CN bond out of the guanine plane or to return $\Theta_{C_2T_1C_6}$ to its initial value raise the energy.

4. Conclusion

In conclusion of this study, it appears that the addition of glyoxal to guanine seems to start at the exocyclic amino group N_2 rather than at the intracyclic imino group N_1. The energy barrier found is lower in the first case (49.7 versus 63.9 kcal/mole). The intermediate product is also more stable when addition occurs first at N_2: -30.7 kcal/mole versus -17.9 kcal/mole when the addition occurs first at N_1.

In order to obtain relatively low energy barriers, one must allow for deformations both at the carbon and at the nitrogen atom. The deformations remain in the final product. Thus replacing an N–H bond by a N–C(OH) bond modifies the hybridization around the nitrogen atom.

The reaction appears to be easier at the exocyclic N_2 amino nitrogen atom for two reasons: 1) the approach to it is easier than to N_1 since the atoms of the glyoxal molecule do not come in such close contact with those of the guanine ring as when the attack is on the imino N_1 nitrogen atom; 2) the deformation of the N_2-amino group is more energy lowering than that of the N_1-imino group. This deformation occurs at an early stage of the reaction and is essential for lowering the energy barrier. The barriers obtained remain nevertheless high. It must be underlined that they represent gas phase reactions but that our results although corresponding to a treatment involving many simplifications are of the same order of magnitude as those obtained in more refined treatments of simpler systems (e.g. [21]). Solvation effects may, of course, have an appreciable influence on the reaction mechanism and the computed energy barriers. We propose to study these effects in a future publication.

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