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Theoretical Study of the Protonation and Tautomerization of Adenosine, Formycin, and Their 2-NH_2 and 2-F Derivatives: Functional Implications in the Mechanism of Reaction of Adenosine Deaminase

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SUMMARY

A quantum chemical study of adenosine, formycin, and their 2-NH_2 and 2-F derivatives is performed. The tautomerism of neutral and protonated species as well as the protonation of adenosine, formycin, and their derivatives are theoretically studied using semiempirical MNDO and AM1, as well as *ab initio* STO-3G methods. Calculations have been performed on a reduced model, in which the ribose moiety has been substituted by a hydroxymethyl group. Results indicate that adenosine is mainly protonated at the N1 atom, whereas formycin can be protonated on N1 or N3, depending on the tautomeric form (N8-H or N7-H). The quantum chemical study of the N1-protonated molecules shows that a second protonation of adenosine is mainly on the N3 atom, whereas formycin can be protonated on N8 or N3, depending on the tautomeric form. On the other hand, results indicate that the protonation of formycin and its derivatives at the N1 atom leads to a change in their tautomeric preference from N7-H to N8-H. The importance of both tautomerism and protonation reactions in the mechanism of action of adenosine deaminase is studied by means of a quantitative structure activity relationships strategy. Significant correlations were found between several electronic parameters and the logarithm of the maximum rate of deamination (log V_m) of the studied compounds. For formycin and its derivatives, it was necessary to consider their N8-H tautomeric forms. The electronic parameters giving good correlations were as follows: energy of the minimum of the *ab initio* molecular electrostatic potential on N1, net charge over purine (pyrazolo-pyrimidine) and pyrimidine rings, and the N1 protonation energy. It must be noted that all these parameters are informative in relation to a proton attack. Adenosine and purine ribosides have been studied largely because of their high biological relevance. They are constitutents of nucleic acids, intermediates in secondary metabolism, neuromodulators, and neurohormones. Their analogues have been extensively used because of their wide range of pharmacological effects (1). Formycin A (Fig. 1) is one of the most studied analogues of adenosine. It is a natural product extracted from *Nocardia interforma* (2) with proven antiviral (3–5), antibiotic (2), immunodepressant (6), antitumor (6), and antimetabolic (5) activities.

The biochemical relevance of tautomerism equilibria of nucleosides and nucleotides is well known. Thus, the existence of unusual tautomeric forms of nucleotides has been related to the induction of mutations (7), and there are several biological effects of nucleosides and nucleotides in which tautomerism plays a key role (1). The protonation of nucleosides and nucleotides is also a very important biological process; it has not only been related to enzymatic reactions but it has also been associated with the stabilization of some forms of DNA (8).

Numerous studies indicate that adenosine is mainly found in its amino form (9–14), whereas other tautomeric forms, such as imino forms, are unstables (13–15). Formycin has been detected crystallographically as the N7-H tautomer (16, 17); nevertheless, ^{13}C NMR studies have demonstrated the existence of a tautomeric equilibrium in solution between N7-H and N8-H forms (18). On the other hand, crystallographic studies of N1-protonated formycin indicate the existence of the N8-H tautomer (19). All studies have described imino forms as unstable (15, 18). It has been also demonstrated that both adenosine and formycin are mainly protonated at the N1 atom (14, 19–22). The tautomeric and protonation equilibria between the most important forms of formycin are shown in Fig. 2.

The *syn* preference around the glycosidic bond of formycin, in constrast to adenosine, which is mainly found in the *anti*-conformation, has been studied (Fig. 3). The *syn*-conformation is maintained in polyformycin, an unusual *syn*-polyribonucleotide (23, 24). However, it has been suggested by experimental (25, 26) as well as theoretical results (25, 27) that the *syn-anti*

ABBREVIATIONS: MEP, Molecular electrostatic potential: LUMO, Lowest unoccupied molecular orbital; MOPAC, Molecular orbital package; MNDO, Modified neglect of diatomic overlap; AM1, Austin model 1; QSAR, quantitative structure activity relationship.

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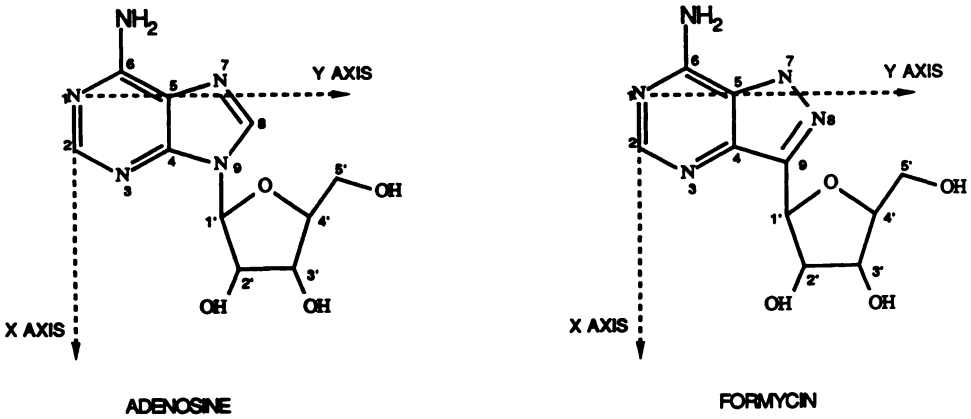

Fig. 1. Representation of adenosine and formycin in the $Z = 0$ plane. Formycin has been numbered following the criterion used for purine ribosides. This criterion has been selected instead of the IUPAC criterion, in order to clarify the discussion of the results.

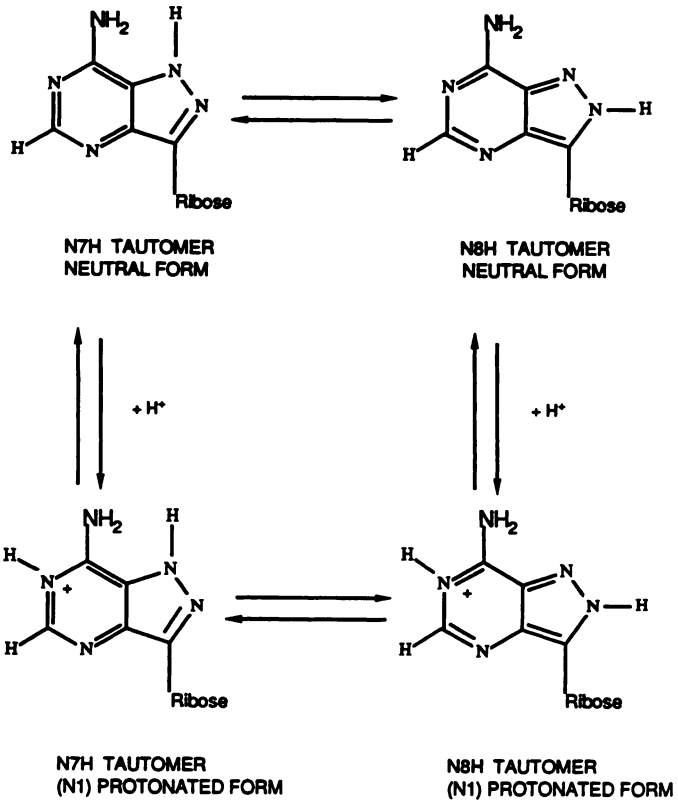

Fig. 2. Equilibria between the main forms of formycin.

conversion of formycin is energetically feasible. This has been suggested to be one of the reasons that explain why formycin is a substrate for several enzymes, the natural substrate of which is adenosine (24, 28). Despite these facts, the reactivity of formycin in comparison with adenosine is not easy to explain for some enzymes.

One of these enzymes is adenosine deaminase (E.C. 3.5.4.4), which cleaves adenosine to produce inosine. This deamination is very important throughout metabolism as demonstrated by cellular dysfunction in diseases such as leukemias (29) and acquired immunodeficiency syndrome (30), in which the enzyme level is altered. Indeed, it has been proven that its genetic defect is associated with severe combined immunodeficiency syndrome (31).

Adenosine deaminase is a very nonspecific enzyme catalyzing hydrolytic displacement of several leaving groups on C6 from purine ribosides and analogues (32, 33). It has been suggested that the chemical mechanism of deamination is likely of the addition-elimination type (33), with direct attack by water on the substrate (33, 34), resulting in the formation of a tetrahedral intermediate (33–35). Recent ^{13}C NMR studies (36) confirm the change from *sp*2 to *sp*3 hybridization of C6 during the deamination reaction. The formation of the tetrahedral intermediate can be divided into at least two elemental steps, (i) protonation of the purine ring at N1 by an active sulfhydryl group (37–40) and (ii) hydroxylation of C6 by means of a water molecule located at the active site (33, 34).

In this paper, the tautomerism of formycin (both neutral and protonated forms) and the protonation of adenosine and formycin, as well as their 2-NH_2 and 2-F derivatives, are studied. The influence of both reactions in the deamination reaction catalyzed by adenosine deaminase is discussed by means of QSAR studies.

Methods

The large size of adenosine makes it difficult to handle from a computational point of view. Thus, several authors (15, 41) have introduced the use of reduced models in which the effect of the ribose moiety is "mimicked" by means of a smaller group. The —CH2OH group has been choosen in this work because, in a previous study,[1] we have demonstrated that this group accurately mimicks the effect of the ribose on the charge and orbital characteristics of the purine ring. The suitability of this simulative model for the study of protonation and tautomerism reactions is discussed below.

The geometry of the molecules were fully optimized (with the only restriction being the planarity of the purine ring) using semiempirical AM1 and MNDO methods (42, 43). Although both methods do not provide quantitatively correct results, they have demonstrated their usefulness for comparative studies of protonation and tautomerization processes in related biological molecules (44, 45).

Semiempirical geometry optimization followed by single *ab initio* STO-3G calculation leads to results very close to those obtained from *ab initio* STO-3G fully geometry optimization for several reactions (46). Hence, *ab initio* calculations were performed using STO-3G basis set and the AM1-optimized geometry.

Protonation energies were calculated by substrating the energy of protonated species from the energy of neutral species. Tautomerization energies were calculated by substracting the energy of one tautomer from that of the other.

It must be noted that the aim of the present study is not to obtain accurate values of the protonation energies, because this implies the use of extended basis sets and probably the introduction of an electronic correlation effect. We intend to obtain reliable relative values of the

[1] Orozco *et al.*, submitted for publication.

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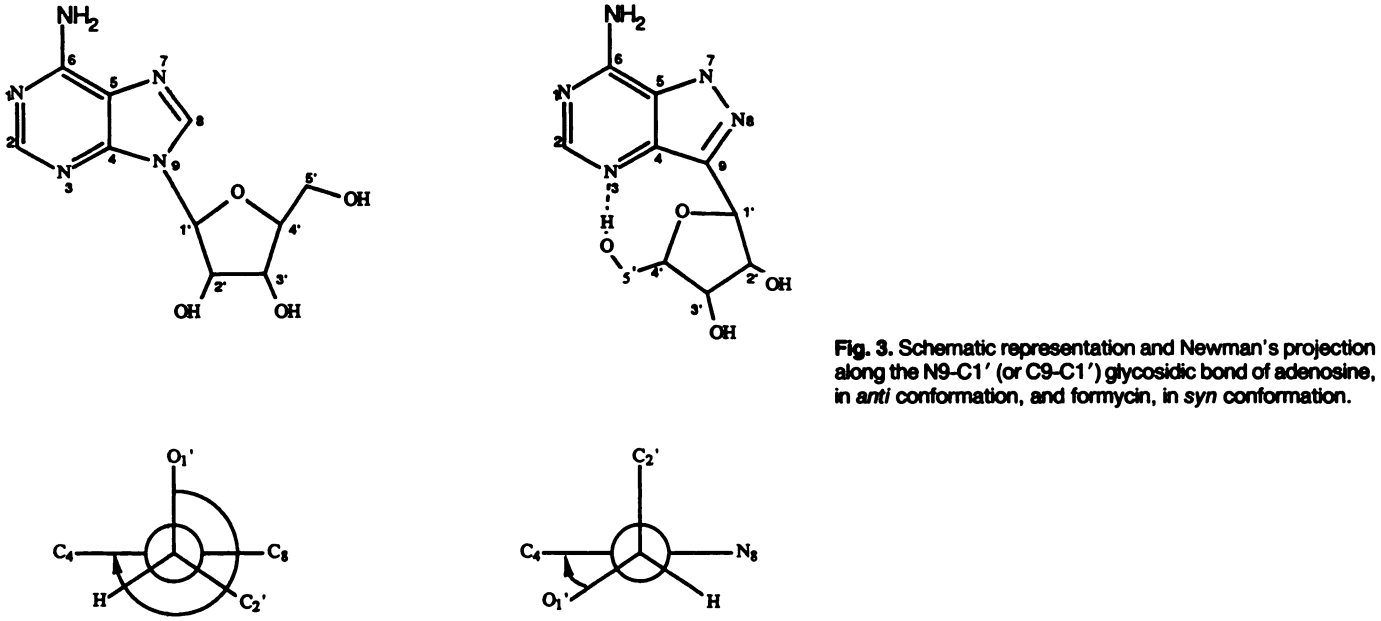

Fig. 3. Schematic representation and Newman's projection along the N9-C1′ (or C9-C1′) glycosidic bond of adenosine, in *anti* conformation, and formycin, in *syn* conformation.

protonation energy in a series of nucleosides and, in this context, semiempirical and *ab initio* STO-3G methods provide results of high enough quality.

Ab initio of the different molecules were calculated using the *ab initio* STO-3G wave function. Minima over the purine ring were detected with an error of less than 0.001 Å. Because MEP gives a measure of the interaction between a positive charge and the whole molecule in a point of the space, it is an excellent measure of the reactivity of the molecule toward a proton attack and therefore it is very useful for the study of protonation reactions (47, 48).

Semiempirical calculations were performed using the MOPAC program (49) in a locally modified version (50). *Ab initio* wave function calculations were performed with the HONDO program (51).

Results

Study of the usefulness of the reduced model in the representation of protonation and tautomerism reactions. As noted above, the ribose moiety has been substituted by a hydroxymethyl group in calculations, according to previous studies[1] in which the usefulness of this model to represent the charge and orbital characteristics of the purine ring was demonstrated. Likewise, in order to examine the usefulness of such a reduced model in computing protonation and tautomerization energies, the energetics of both reactions for 2-NH_2-formycin considering the whole molecule and the simulative model have been studied by means of the AM1 method (see Table 1). Results clearly show the ability of the simulative model to reproduce the values of protonation and tautomerization energies obtained from the calculation using the whole molecule.

Study of protonation of adenosine, formycin, and derivatives. The minima of the *ab initio* MEP over the nitrogen atoms of the aromatic ring of C8-H tautomers of adenosine and derivatives and both N7-H and N8-H tautomers of formycin and derivatives have been calculated. The position of the minima as well as their energetic values are shown in Table 2 (for reference coordinates, see Fig. 1). Likewise, the energy of the protonation over N1 of the molecules, calculated with different methods, are shown in Tables 3, 4, and 5.

TABLE 1

Comparison of AM1 protonation and tautomerism energies of 2-NH_2 formycin calculated from the whole molecule or the reduced model used in this study

	Protonation Energy		Tautomerization Energy	
	N8-H Tautomer	N7-H Tautomer	Neutral forms	Protonated forms
		eV		
Reduced model	−7.396786	−7.243145	0.038049	0.191692
Full molecule	−7.363432	−7.139147	0.047269	0.271561

The comparison of *ab initio* MEP energy minima on N1 and *ab initio* STO-3G and semiempirical protonation energies points out that all these parameters are highly correlated (Table 6). Thus, any one of them supplies similar information in comparative studies.

The energy values of the MEP minima point out that N1 is the most favorable atom to be protonated on the aromatic ring in adenosine and its derivatives, as well as in N8-H-formycin. Nevertheless, N7-H-formycin, as well as the 2F- and 2NH2-derivatives of formycin (in both N7-H and N8-H tautomeric forms), shows a preference for N3 protonation.

These results are in good agreement with the existence of both N1 and N3 protonated forms of formycin, previously detected in solution (1), in contrast to the existence of an unique N1 protonated form of adenosine. The most favorable protonation site being N1 (1, 19) in formycin can be easily justified by the steric hindrance of the ribose moiety over N3, due to the *syn* conformation around the glycosidic bond of formycin (see below).

MEP energy minima, as well as protonation energies, confirm, as expected by chemical intuition, that the presence of an

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TABLE 2

Position and energetic value of the *ab initio* MEP minima on the aromatic ring of formycin, adenosine, and their 2-NH_2 and 2-F derivatives

Compound	Tautomeric Form	Atom	Coordinates of the minimum x	y	z	Energy
						kcal/mol
Formycin	N8-H	N1	−0.502	−0.894	0.000	−92.5630
		N3	3.262	0.874	0.000	−91.6032
		N7	−1.082	4.234	0.000	−68.8734
	N7-H	N1	−0.504	−0.902	0.000	−83.0746
		N3	3.248	0.893	0.000	−85.4847
		N8	1.081	5.544	0.000	−72.9763
2-F-Formycin	N8-H	N1	−0.498	−0.904	0.000	−87.1018
		N3	3.291	0.925	0.000	−88.9332
		N7	−1.010	4.233	0.000	−65.8106
	N7-H	N1	−0.503	−0.908	0.000	−79.3763
		N3	3.286	0.936	0.000	−82.7529
		N8	1.060	5.554	0.000	−71.7171
2-NH_2-Formycin	N8-H	N1	−0.506	−0.882	0.000	−89.3280
		N3	3.262	0.995	0.000	−96.6183
		N7	−1.159	4.208	0.000	−71.8367
	N7-H	N1	−0.511	−0.886	0.000	−84.2985
		N3	3.250	1.027	0.000	−91.6018
		N8	0.974	5.565	0.000	−79.0189
Adenosine	C8-H	N1	−0.514	−0.891	0.000	−88.6906
		N3	3.251	0.861	0.000	−81.7397
		N7	−1.102	4.232	0.000	−81.4987
2 F-Adenosine	C8-H	N1	−0.511	−0.898	0.000	−85.0150
		N3	3.286	0.902	0.000	−78.7296
		N7	−1.109	4.226	0.000	−79.8796
2-NH_2-Adenosine	C8-H	N1	−0.511	−0.879	0.000	−89.7139
		N3	3.251	0.995	0.000	−84.7536
		N7	−1.185	4.200	0.000	−86.5550

TABLE 3

Protonation and tautomerization energies of formycin, adenosine, and their 2-NH_2 and 2-F derivatives calculated using the AM1 method

Compound	Tautomer	Protonation Energy	Tautomerization Energy Neutral forms	Protonated forms
		eV		
Adenosine	C8-H N7-H	−7.354501	0.799539	
2-F-Adenosine	C8-H N7-H	−7.078557	0.826098	
2-NH_2-Adenosine	C8-H N7-H	−7.468322	0.807076	
Formycin	N8-H N7-H	−7.324433 −7.092683	0.064708	0.296462
2-F-Formycin	N8-H N7-H	−7.059733 −6.825213	0.078441	0.312960
2-NH_2-Formycin	N8-H N7-H	−7.396786 −7.243145	0.038049	0.191692

amino group at the 2-position leads to an increase in the ability for protonation on N1. In contrast, the presence of a fluorine group leads to a decrease in this ability.

The comparison of MEP energy minima over N1 between adenosine and its analogues and formycin and its analogues points out that the protonation on N1 is easier in adenosine and its derivatives than in the N7-H form of formycin and its derivatives but more difficult than in the N8-H form of formycin and its derivatives.

The second protonation for both adenosine and formycin, as well as their analogues, has been studied, assuming that the first protonation occurs on N1 in accordance with the results discussed above. For this purpose, the minima of the *ab initio* MEP on N3 and N7 (or N8) of molecules protonated at the N1 atom have been calculated (Table 7).

TABLE 4

Protonation and tautomerization energies of formycin, adenosine, and their 2-NH_2 and 2-F derivatives calculated using the MNDO method

Compound	Tautomer	Protonation Energy	Tautomerization Energy Neutral forms	Protonated forms
			eV	
Adenosine	C8-H N7-H	−7.489026	1.409131	
2-F-Adenosine	C8-H N7-H	−7.089550	1.436523	
2-NH_2-Adenosine	C8-H N7-H	−7.505089	1.417128	
Formycin	N8-H N7-H	−7.523080 −7.241866	0.034510	0.315645
2-F-Formycin	N8-H N7-H	−7.119742 −6.840251	0.065955	0.345451
2-NH_2-Formycin	N8-H N7-H	−7.319887 −7.180287	0.045641	0.185243

TABLE 5

Protonation and tautomerization energies of formycin, adenosine, and their 2-NH_2 and 2-F derivatives calculated using the *ab initio* STO-3G method

Compound	Tautomer	Protonation Energy	Tautomerization Energy Neutral forms	Protonated forms
			eV	
Adenosine	C8-H N7-H	−12.485854	1.297290	
2-F-Adenosine	C8-H N7-H	−12.267684	1.357992	
2-NH_2-Adenosine	C8-H N7-H	−12.701305	1.326039	
Formycin	N8-H N7-H	−12.634582 −12.158076	−0.196659	0.279823
2-F-Formycin	N8-H N7-H	−12.340411 −11.940362	−0.119841	0.280209
2-NH_2-Formycin	N8-H N7-H	−12.648566 −12.403969	−0.094275	0.150319

Results clearly establish that a second protonation on adenosine and its analogues is much more difficult than the first protonation and that N7 is the less unfavored atom to pick up a proton, as suggested by previous *ab initio* STO-3G calculations (52), as well as by experimental evidence (21). N8 is the most suitable atom for a second protonation in N7-H-formycin and derivatives. Nevertheless, it is interesting to note that N3 is the mot probable target atom for a second protonation of N8-H-formycin and derivatives (see Table 7).

Study of the tautomerism of formycin and derivatives. The tautomeric equilibrium N7-H ↔ N8-H of formycin and its derivatives in neutral and N1-protonated forms has been studied. For comparison purposes, the N7-H form of adenosine (this form implies charge separation and it is presumably unstable), which is structurally similar to the N7-H tautomer of formycin, has also been calculated.

The comparison of the tautomeric energies obtained from AM1 and MNDO methods with those obtained from the *ab initio* STO-3G method indicates that MNDO and AM1 methods are suitable for comparative purposes (see Table 6). Nevertheless, both methods fail in the prediction of the most stable tautomeric forms of formycin and analogues, because they find that N8-H forms are more stable than N7-H forms, when

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TABLE 6

Statistical parameters of the correlations between protonation energies calculated semiempirically and *ab initio*; between energy of the MEP minimum on N1 and protonation energy; and finally, between tautomerization energy calculated semiempirically and *ab initio*

Independent Variable	Dependent Variable	Regression Coefficient	Student's *t*	Confidence Level
MNDO Protonation Energy	STO-3G Protonation Energy	0.870	4.600	$p < 0.001$
AM1 Protonation Energy	STO-3G Protonation Energy	0.956	8.566	$p < 0.0001$
MNDO Protonation Energy	MEP Minimum Energy (N1)	0.878	4.850	$p < 0.001$
AM1 Protonation Energy	MEP Minimum Energy (N1)	0.838	4.064	$p < 0.0025$
MEP Minimum Energy (N1)	STO-3G Protonation Energy	0.931	6.737	$p < 0.0001$
AM1 Tautomerism Energy	STO-3G Tautomerism Energy	0.998	32.04	$p < 10^{-6}$
MNDO Tautomerism Energy	STO-3G Tautomerism Energy	0.999	68.54	$p < 10^{-7}$

TABLE 7

Position and energetic values of the *ab initio* MEP on the aromatic ring in formycin, adenosine, and their derivatives protonated at N1

Compound	Tautomeric Form	Atom	Coordinates of the Minimum			Energy
			x	*y*	*z*	
						kcal/mol
Formycin	N8-H	N3	3.275	0.938	0.000	4.5823
		N7	−1.226	4.227	0.000	28.0671
	N7-H	N3	3.266	0.957	0.000	15.5259
		N8	0.933	5.575	0.000	8.6886
2-F-Formycin	N8-H	N3	3.313	0.983	0.000	6.4254
		N7	−1.230	4.223	0.000	29.5122
	N7-H	N3	3.303	1.000	0.000	18.0739
		N8	0.930	5.585	0.000	9.2339
2-NH_2-Formycin	N8-H	N3	3.254	1.094	0.000	−3.2061
		N7	−1.318	4.197	0.000	22.3888
	N7-H	N3	3.244	1.115	0.000	8.8955
		N8	0.827	5.612	0.000	0.5912
Adenosine	C8-H	N3	3.263	0.917	0.000	21.3748
		N7	−1.237	4.218	0.000	7.3047
2-F-Adenosine	C8-H	N3	3.305	0.961	0.000	24.5089
		N7	−1.243	4.215	0.000	7.9718
2-NH_2-Adenosine	C8-H	N3	3.257	1.086	0.000	9.6609
		N7	−1.328	4.190	0.000	1.0780

several experimental results point out that N7-H is the most stable tautomer of formycin (16–18). *Ab initio* calculations indicate that N7-H is the most stable tautomer, in agreement with experimental data. Therefore, we must conclude that *ab initio* methods must be used in order to study the tautomeric preference of formycin and derivatives and that semiempirical AM1 and MNDO methods, although they can be useful for comparative purposes (see Table 6), do not provide correct results for the tautomeric preference of these molecules.

Although *ab initio* STO-3G results show that N7-H is the most stable tautomer for formycin and derivatives, the differences between the energy of both tautomers are small (between 2.7 and 4.5 kcal/mol). When formycin and its derivatives are protonated on N1, the N8-H tautomer notably increases its stability (between 3.2 and 6.4 kcal/mol in semiempirical calculations and 10.9 and 5.6 kcal/mol in STO-3G calculations). As a consequence, there is a change in the tautomeric preference, from the N7-H to the N8-H tautomer, when formycin and its derivates are protonated on the N1 atom (*ab initio* results suggest differences between 3.4 and 6.4 kcals/mol). These results are in good agreement with the surprising existence, detected in crystallographic studies, of the N8-H tautomer in N1-protonated formycin (19).

QSAR between the electronic characteristics of formycin, adenosine, and derivatives and the rate of deamination by adenoside deaminase. The six compounds selected in this study are substrates of adenosine deaminase (53) and, in spite of their great structural similarity, their maximum rates of deamination (V_m) are quite different (Table 8).

As noted above (see the introduction), there is not any evident explanation for the fact that formycin is deaminated much faster than the natural substrate, adenosine, by adenoside deaminase. This fact seems more difficult to understand, when considering that formycin is mainly in the N7-H tautomeric form, whereas adenosine is not in this tautomeric form.

If we try to correlate electronic characteristics of adenosine and its analogues and those of N7-H formycin and its analogues (Tables 2, 3, and 8) with their respective log (V_m) of deamination, no significant correlations are obtained. However, when formycin and its derivatives are considered in the N8-H tautomeric form, significant correlations between several electronic parameters of the six studied compounds [energy of the minimum of the MEP on N1, net charge over purine (or pyrazolo-pyrimidine) and pyrimidine rings, or N1 protonation energy] and their log (V_m) of deamination by adenosine deaminase are found (see Table 9 and Fig. 4). It should be noted that all these parameters are informative, in relation to a proton attack. The introduction of parameters of E_{LUMO}, or charge over C6 of both neutral and protonated species, which could be informative if an hydroxyl attack was the rate-limiting step, did not lead to significant increases in the regression coefficients. These results clearly establish a correlation between protonation and the deamination reaction, in such a way that an increase in the feasibility for the protonation on N1 implies an increase in the rate of deamination catalyzed by adenosine deaminase. Results also suggest that the deamination rates of these compounds are not correlated with parameters informative of the hydroxyl attack over the C6 atom.

Discussion

Comparison of *ab initio* STO-3G and semiempirical methods shows that both methods provide similar information in comparative studies. Nevertheless, semiempirical methods fail in the prediction of more stable tautomeric forms and, therefore, in studies of tautomeric preference, *ab initio* methods must be used. The agreement of the results obtained from *ab initio* STO-3G calculations with experimental data is excellent and, therefore, we can conclude that *ab initio* STO-3G is a suitable method for the kind of studies performed here.

Results suggest that adenosine is mainly protonated at the N1 atom, as previously described (14, 20–22). Nevertheless, formycin and its derivatives can be protonated on N1 or N3, depending on their tautomeric form. Thus, it seems that the protonation on N1 is most favorable for N8-H-formycin, whereas N3 protonation is more likely to occur for N7-H-formycin.

As noted above (see the introduction), formycin is usually found in the *syn* conformation and a hydrogen bond between

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TABLE 8

Charges on N1, C6, pyrimidine, and purine (or pyrazolopyrimidine) rings calculated by AM1, MNDO, and *ab initio* STO-3G methods and maximum rates of deamination (relative to 100 of adenosine) of adenosine, formycin, and their derivatives.

Values of V_{max} are taken from Ref 53.

Compound	Tautomeric Form	Calculation Method	Q_{N1}	Q_{C6}	Q_{pyr}	Q_{pur}	V_{max}
Formycin	N8-H	*Ab initio* STO 3G	−0.3114	0.2574	−0.1240	−0.4150	
		AM1	−0.2791	0.2425	−0.4617	−0.7451	919
		MNDO	−0.3792	0.3345	−0.3016	−0.5627	
	N7-H	*Ab initio* STO 36	−0.2881	0.2390	−0.0655	−0.3797	
		AM1	−0.2405	0.2089	−0.3986	−0.7209	
		MNDO	−0.3449	0.3026	−0.2258	−0.5463	
2-F-Formycin	N8-H	*Ab initio* STO 3G	−0.3230	0.2665	0.0592	−0.2288	
		AM1	−0.2948	0.2678	−0.2042	−0.4716	3.9
		MNDO	−0.3761	0.3538	−0.0996	−0.3448	
	N7-H	*Ab initio* STO 36	−0.3050	0.2485	0.1182	−0.1932	
		AM1	−0.2619	0.2377	−0.1347	−0.4426	
		MNDO	−0.3477	0.3245	−0.0179	−0.3259	
2-NH_2-Formycin	N8-H	*Ab initio* STO 3G	−0.3318	0.2644	−0.0083	−0.3152	
		AM1	−0.3182	0.2728	−0.3313	−0.6475	287
		MNDO	−0.3855	0.3518	−0.2318	−0.5085	
	N7-H	*Ab initio* STO 36	−0.3200	0.2454	0.0428	−0.2867	
		AM1	−0.2980	0.2460	−0.2814	−0.6278	
		MNDO	−0.3632	0.3251	−0.1627	−0.4933	
Adenosine	C8-H	*Ab initio* STO 3G	−0.3017	0.2513	−0.0094	−0.4060	
		AM1	−0.2698	0.2448	−0.3953	−0.7752	100
		MNDO	−0.3776	0.3420	−0.2075	−0.6135	
2-F-Adenosine	C8-H	*Ab initio* STO 3G	−0.3188	0.2599	0.1734	−0.2223	
		AM1	−0.2911	0.2703	−0.1370	−0.5040	0.002
		MNDO	−0.3805	0.3618	−0.0026	−0.3977	
2-NH_2-Adenosine	C8-H	*Ab initio* STO 3G	−0.3351	0.2579	0.0968	−0.3135	
		AM1	−0.3290	0.2793	−0.2872	−0.6822	25
		MNDO	−0.4196	0.3709	−0.1322	−0.5517	

TABLE 9

Statistical parameters of the significant (confidence level greater than 95%) correlations between the log(V_m) of deamination and different electronic parameters of adenosine, formycin, and their derivatives

Independent Variable	Regression Coefficient	Student's *t*	Confidence Level
STO-3G Protonation Energy	0.801	2.681	$p < 0.05$
MEP Minimum Energy (N1)	0.890	3.880	$p < 0.01$
Charge over Pyrimidine Ring	0.872	3.561	$p < 0.015$
Charge over Purine Ring	0.785	2.543	$p < 0.05$

N3 and H(05′) is described (1, 25–27). Despite the similarity between adenosine and formycin, adenosine is found in the *anti* conformation and no hydrogen bond is detected. We can expect that an increase in the basicity on N3 favors the existence of the hydrogen bond. Because our results indicate that the N3 MEP minimum is deeper in formycin than in adenosine, it is not surprising that the formation of a hydrogen bond is more likely to be established in formycin than in adenosine. On the other hand, as noted above, the existence of this hydrogen bond (and consequent stabilization of the *syn* conformer) leads to a decrease in the ability for protonation of N3, due to steric factors, and, therefore, the predominant existence of the N1-protonated form in formycin is well understood.

Ab initio results show that formycin and its derivatives are more stable in their N7-H tautomeric forms than in their N8-H forms, although energetic differences are small, in good agreement with experimental data (18). This tautomeric preference changes when formycin and its derivatives are protonated at N1; in that case, the N8-H tautomer is the most stable form. It should be noted that the protonation on N1 is easier for formycin and its derivatives in their N8-H form than in the N7-H form; until now this fact has not been taken into consideration when kinetics of tautomerization are studied (54).

From another point of view, N8-H-formycin and its derivatives show more ability of protonation on N1 than do adenosine and its derivatives. In contrast, protonation on N1 is more difficult for N7-H formycin than for adenosine.

The rate of deamination of formycin by adenosine deaminase is 10-fold greater than for adenosine. Nevertheless, its apparent K_m value is 20-fold greater (53, 55). 2-NH_2 and 2-F analogues of formycin and adenosine show similar characteristics (53). This suggests that, although recognition of formycin and derivatives by adenosine deaminase is difficult, once it occurs the deamination is quickly performed. It has been suggested that a lone pair in the N7 position is necessary for the recognition and further deamination by adenosine deaminase. The fact that tubercydin (56) is not a substrate for adenosine deaminase, as well as the high rate of deamination of 8-methyl-formycin in contrast to the poor substrate properties of 7-methyl-adenosine (5, 57), agrees with this hypothesis. Consequently, formycin and its derivatives must be recognized by adenosine deaminase in their N8-H tautomeric form. Our results indicate that, in the neutral form, N8-H is not the most stable tautomeric form of formycin and its derivatives; thus, a tautomeric change is required and the high apparent K_m values of these compounds are not surprising.[2] It is interesting to note that this tautomeric change causes an important increase in basicity of N1. If we

[2] It can be easily demonstrated that the value of the apparent K_m, when a tautomerism equilibrium exists, can be calculated according to:

$$K_{mapp} = K_m (1 + K_T) \quad (1)$$

where K_T is the equilibrium constant for the tautomerization between N8-H and N7-H forms.

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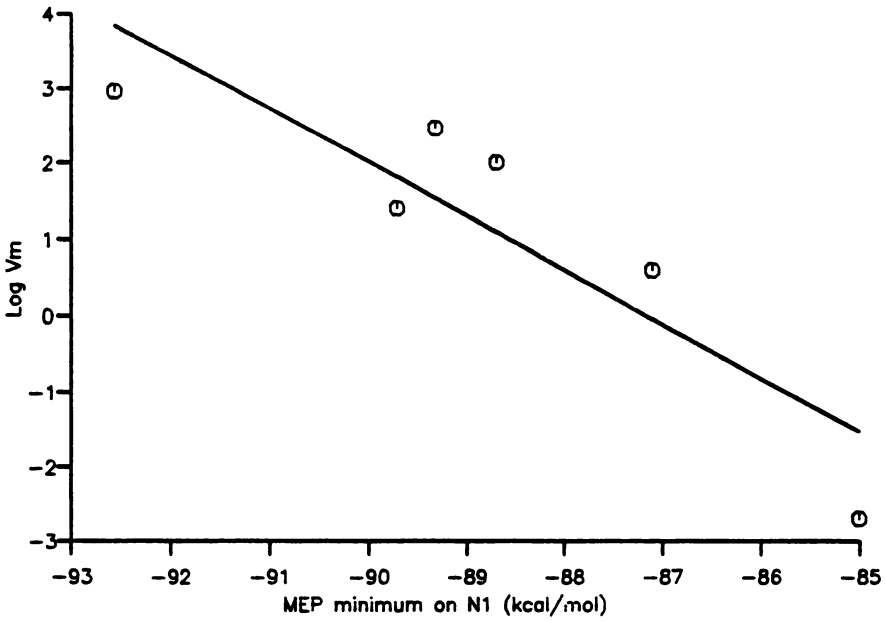

Fig. 4. Plot of the correlation found between the log (V_m) and the *ab initio* STO-3G MEP on N1 of adenosine, N8-H-formycin, and their 2-F and 2-NH_2 derivatives.

assume that the protonation reaction is before the hydroxyl attack, as it has been experimentally (37, 39) and theroretically[1] suggested, QSAR studies of neutral and protonated species will be a powerful tool to determine the rate-limiting step of the reaction. Our results clearly demonstrate that there are significant correlations between parameters that are informative about proton attack and the rate of deamination. Therefore, although conclusions are drawn from the study of six analogues of adenosine and formycin, there is strong evidence indicating that the protonation on N1 could contribute to the rate-limiting step of the deamination reaction catalyzed by adenosine deaminase.

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