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Inhibition mechanism of flavin by deprenyl as an acetylenic irreversible inhibitor*

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Abstract. Possible inhibition mechanisms of flavin (isoalloxazine) with (−)-deprenyl as an acetylenic irreversible inhibitor have been investigated in detail by ab initio methods with the 6-31G* basis set through the simplified model compounds 3-formyl-2-imino-1-hydropyrazine and propargylamine. The resulting compounds have been verified by calculations with the 3-21G basis set using flavin itself and the model of (−)-deprenyl for confirmation of the reactions through the simplified models. Two cyclic O4,N5- and C4a,N5-covalent adducts have been found. The latter was the most stable and was considered to be the final irreversible product. The intermediates in the reaction, the acyclic C4a- or N5-allenic compounds and their hydrogen-transferred cyanine-type compounds, are in agreement with the results of experimental photochemical reactions. In most of the reaction processes, hydrogen migration played an important role.

Key words: Flavin – Deprenyl – Irreversible inhibitor – Acyclic adduct – Cyclic adduct – Hydrogen migration

1 Introduction

(−)-Deprenyl [1], (*R*)-*N*, α-dimethyl-*N*-propynylbenzeneethanamine, is an acetylenic irreversible inhibitor [2, 3] of type B monoamine oxidase (MAO-B) [4–6] and has been widely used in clinical treatment of Parkinson's disease [7] in combination with (−)-DOPA as a dopamine precursor. Parkinson's disease is a progressive disorder which is characterized by the deficiency of dopamine as a chemical transmitter due to degeneration of dopaminergic neurons in the brain. Its symptoms are ameliorated by dopaminergic therapy based on treatment with (−)-DOPA. (−)-Deprenyl has the ability to potentiate and prolong the efficacy of (−)-DOPA, since (−)-deprenyl inhibits MAO-B oxidation of dopamine in the brain.

MAO-B contains a covalently linked flavin coenzyme, flavine-adenine-dinucleotide (FAD), in the active site and the biocatalytic entity is the flavin nucleus [8] in FAD. The irreversible inhibition of MAO-B by acetylenic inhibitors such as (−)-deprenyl has been accepted as being caused by the formation of an irreversible covalent bond [2, 9a, 9b, 10] between the inhibitor and flavin rendering flavin inactive. There have been a number of enzymatic [9c, 11] and nonenzymatic [12] investigations of inhibitions which involve the formation of inhibitor flavin adducts. Most of the nonenzymatic investigations are concerned with photochemical reactions [3, 13, 14] based on the radical mechanism of the single electron-transfer route [10, 12]. However, none of the inhibitions has been characterized from a theoretical point of view. Thus we considered that theoretical investigations [15] were needed as a basis for understanding more precisely the experimental results as well as for providing an insight into the enzymatic function mechanisms not only of the inhibitions but also of the reactivities of the flavin ring. It is known that in actual biological systems, many reactions may occur in aqueous solution. However, in the MAO-B active site, the reactions are considered to proceed under nonaqueous conditions as is the case for the active site in other flavoproteins with known structures. Therefore we have not discussed the solution effect here.

In the present paper we describe our quantum chemical investigations of the irreversible inhibition mechanism of the flavin ring by (−)-deprenyl using a simplified model system. We have manipulated most of the calculations for the reaction routes in the singlet states. Furthermore we have analyzed the relationship between the reaction routes in the singlet states and in the radical states in consideration of the results of experimental photochemical reactions. As models, 3-formyl-2-imino-1-hydropyrazine (3) and propargylamine (4) have been chosen for isoalloxazine (1), the simplest flavin ring, and for (−)-deprenyl (2), respectively. Our study in terms of the frontier molecular

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orbitals of the simplest models, (3) and (4), has shown the formation of two cyclic covalent adducts which would be energetically stable. One was the O4,N5-adduct (5) and the other was the C4a,N5-adduct (6) (Fig. 1). We have discovered interesting and unique reaction mechanisms which give the two cyclic products.

2 Method

The Gaussian94 program [16] was used for all calculations using on NEC SX-4 computer. The geometries of all singlet states were fully optimized by the restricted Hartree-Fock method [17]. Detailed calculations using the 6-31G* basis set [18] on the "small" system, system A, which includes monocyclic flavin model (3) were checked with calculations using the 3-21G basis set [19] on the "large" system, system B, which includes tricyclic isoalloxazine (1). Thus all the results in system A were confirmed qualitatively as being produced in system B with the real flavin ring. The reaction mechanisms were examined in accordance with dynamic aspects along the intrinsic reaction coordinates (IRC) [20]. Some radical states of the products in system A and of all reactants were also fully calculated by the unrestricted Hartree-Fock (UHF) method [21] using the 6-31G* basis set. The atomic charges were calculated by Mulliken population analysis [22]. Frequency analysis in the Gaussian94 program was employed for verification of the transition states.

3 Geometries and electronic states of the reactants

Isoalloxazine (1), the simplest flavin ring, and (−)-deprenyl (2) were studied as reactants. For easier understanding of the reaction mechanisms, their simplified models, (3) and (4), respectively, were considered. The redox activities of the flavin ring mainly occur at atoms N5, C4a, C10a and N1, and the active site of (2) is considered to be the acetylenic part.

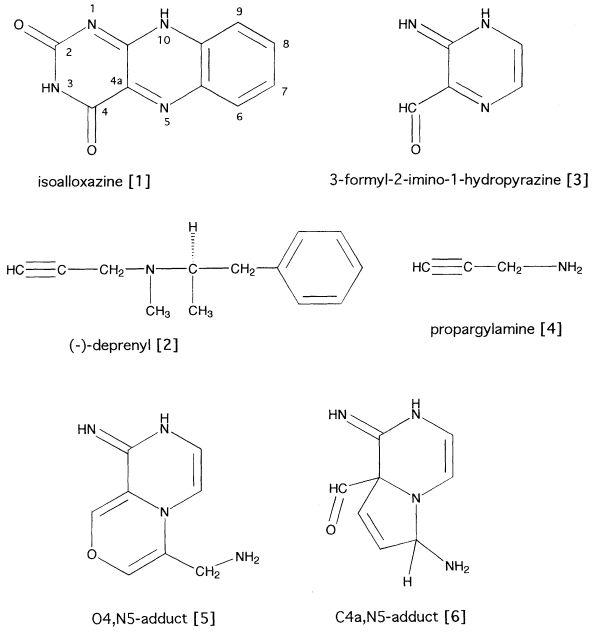


Fig. 1. Reactants, models and products

The optimized configurations of molecules (1)–(4) together with some related bond lengths and angles are shown in Fig. 2. The oxidized state of (1) has a planar conformation in agreement with the result of Zheng and Ornstein [23]. The conformational properties of (1) and (2) have been ascertained to be similar to those of their models (3) and (4), respectively.

The Frontier molecular orbitals of (1), (3) and (4) are shown in Fig. 3. For the flavin case, they are very similar for (1) and its simplified model (3). For (2), the actual HOMO and LUMO are on the phenyl ring; however, the reaction takes place on the acetylenic part of this molecule. In the dense energy levels around the frontier orbitals, the proper orbitals on the acetylenic part were found with energy levels −0.382 and 0.208 a.u., corresponding well to the respective HOMO (−0.377 a.u.) and LUMO (0.198 a.u.) of the simplified model (4). Therefore (3) and (4) have been regarded as suitable models for (1) and (2).

The energy levels of the LUMOs of (1) and (3) are relatively low and it is well-understood that for (1) and (3) electron transfer into these orbitals is easy. The energy difference between the LUMO of (3) and the HOMO of (4) is 0.439 a.u.; while that between the HOMO of (3) and the LUMO of (4) is 0.513 a.u.; the former interaction is therefore considered to be favorable.

The anion radical states of (1) and (3) were also studied. Some related bond lengths, the Mulliken charges and the spin densities are given in Table 1. It is found that the bond lengths of N5—C4a are elongated and those of C4—C4a, C4a—C10a and N5—C5a are shortened, which means that atoms N5 and C4a in the anion-radical state are more active than those in singlet states. This can also be predicted by comparison of the LUMOs of the singlet states of (1) and (3) (0.020 and 0.062 a.u.) with the SOMOs of the anion-radical states of (1) and (3) (−0.108 and −0.063 a.u.). In addition, the phases of the SOMOs of the anion-radical states of (1) and (3) are very similar to those of the LUMOs of the singlet states. For spin densities of the anion-radical states of (1), which agree well with the results of Zheng and Ornstein [23], the high spin densities on the atoms of the benzene ring seem to cancel each other, for example, between C5a and C9a (Table 1); the spin densities are then mainly located on atom N5. This is also the case with (3) which does not have a benzene ring.

The total energies of the anion-radical states of (1) and (3) are 33.22 and 2.69 kcal/mol lower than those of the singlet states, respectively. It is noticeable that (1) is stabilized by gaining one electron, confirming a well-known fact that the flavin radical anion is enzymatically stable. Even its involvement in nonenzymatic reactions has been proposed [10, 14, 24]. The large difference between the stabilization energies for the anion-radical states of (1) and (3) is due to the difference between the tricyclic and monocyclic ring conformations and this implies the involvement of the flavin ring.

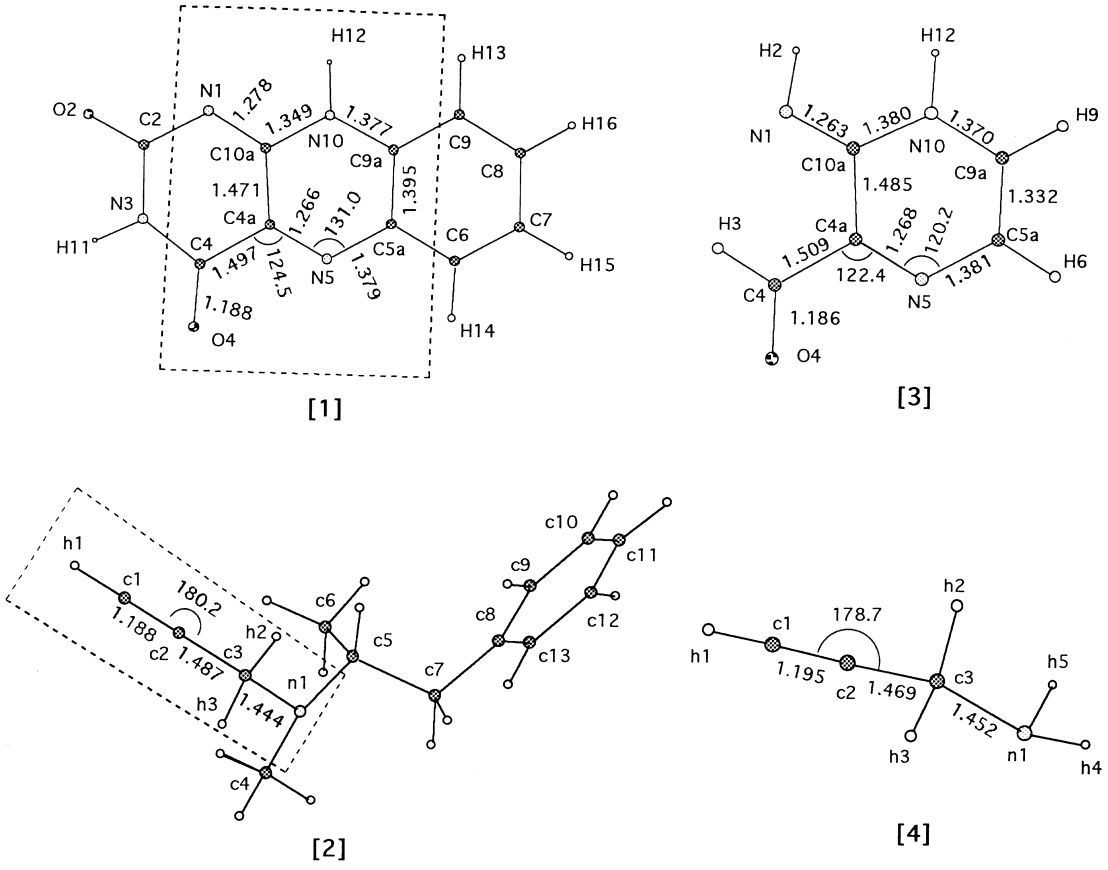


Fig. 2. Optimized geometries of *isoalloxazine* (*1*), (−)-*deprenyl* (*2*), and their simplified models (*3*) and (*4*). The *numbers* are the bond lengths (Å) and bond angles (degree)

The lowest triplet states of (1) and (3) were also examined (Table 1), because their involvement has been observed in experimental photochemical reactions [13a, 13c, 14]. The conformations were flat for all the singlet, triplet and anion-radical states, even though the bond lengths and angles changed and these conformational changes in the triplet states were similar to the ones in the anion-radical states. The highest SOMOs of the triplet states have similar phases to the LUMOs of the singlet states and their energy levels are −0.313 and −0.294 a.u. for (1) and (3), respectively. The spin densities of the triplet states of (1), which agree quite well with the results of Song and coworkers [25] except for the ones on the benzene ring, are mostly on N5 and C4a as for the anion-radical states. However, this is not so clear in (3) which needs further cancellations between C4 and O4, and between C10a and N1. The total energies of the triplet states of (1) and (3) are 47.23 and 19.45 kcal/mol higher than those of the singlet states, respectively. It is more difficult for the tricyclic system to get into the triplet state than for the monocyclic system. This is the opposite of the anion-radical state case. For all states the atomic charges on atoms C4, C5a and C9a are more localized in (1) than in (3) and all the nitrogen and oxygen atoms are always negatively charged.

4 Reactions

4.1 Reaction I: Diels-Alder reaction

According to the above considerations of the molecular orbitals, a favorable interaction is predicted between the LUMO on atoms N5 and O4 of (3) and the HOMO on c1 and c2 in the triple bond of (4). Thus the cyclic product (5) and the corresponding transition state TS-1 for which the activation energy was 64.05 kcal/mol, have been found. This is a one-step Diels-Alder cycloaddition reaction, making a new six-membered ring consisting of atoms N5, C4a, C4, O4, c2 and c1. Figure 4 represents the energy curve along the IRC. In Fig. 4 it can be seen that (4) approaches almost perpendicularly to (3) in the transition state. The torsion angle C4a-N5-c1-c2 was 53.9°, and that the cyclic product (5) made this angle almost zero, becoming coplanar with the plane of (3).

The total energies of (5) and of the corresponding molecule in system B are 24.11 and 27.40 kcal/mol lower than those of the reactants, (3) and (4) in system A and (1) and (4) in system B, respectively, and there is little difference in their conformations. Experimental results on this type of product have been reported [26].

4.2 Reaction II: hydrogen transfer and acyclic allenic adduct

In the interaction between the LUMO of (3) and the HOMO of (4), another favorable connection was

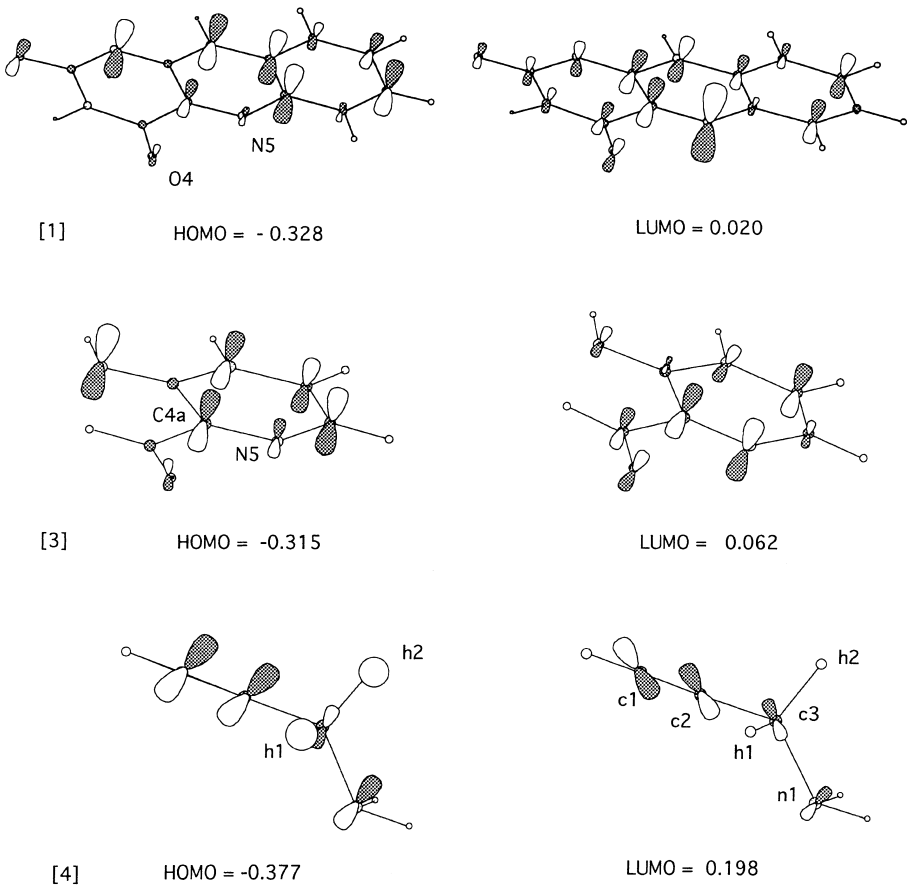


Fig. 3. The Molecular orbitals (MOs) of the reactants. The energy levels are in atomic units

Table 1. The bond lengths (Å), Mulliken atomic charges and spin densities (electron unit) of flavin (1) and its model (3)

	(1)			(3)		
	Singlet	Triplet	Anion radical	Singlet	Triplet	Anion radical
Bond lengths						
C4—C4a	1.497	1.444	1.440	1.509	1.441	1.423
C4a—N5	1.266	1.336	1.374	1.268	1.433	1.377
C4—O4	1.188	1.200	1.210	1.186	1.219	1.221
Ca—C10a	1.471	1.434	1.401	1.485	1.434	1.454
N5—C5a	1.379	1.356	1.344	1.381	1.292	1.332
Mulliken charges						
C4	0.871	0.887	0.829	0.329	0.246	0.294
C4a	0.059	0.003	−0.082	0.115	0.124	−0.060
N5	−0.498	−0.434	−0.583	−0.463	−0.508	−0.537
O4	−0.547	−0.599	−0.672	−0.457	−0.433	−0.650
C5a	0.174	0.286	0.240	−0.073	0.051	−0.003
C10a	0.735	0.763	0.697	0.637	0.582	0.636
N10	−0.931	−0.971	−0.951	−0.858	−0.842	−0.864
C9a	0.423	0.384	0.369	0.117	0.019	0.012
N1	−0.713	−0.725	−0.794	−0.746	−0.616	−0.832
Spin density						
C4		−0.069	0.013		−0.543	−0.023
C4a		0.338	−0.029		0.926	0.055
N5		1.446	0.739		0.529	0.663
O4		0.119	0.044		0.542	0.168
C5a		−0.742	−0.711		−0.443	−0.661
C10a		−0.049	0.063		−0.638	−0.039
N10		0.043	0.064		0.092	0.066
C9a		0.712	0.648		0.836	0.789
N1		0.131	0.014		0.780	0.020

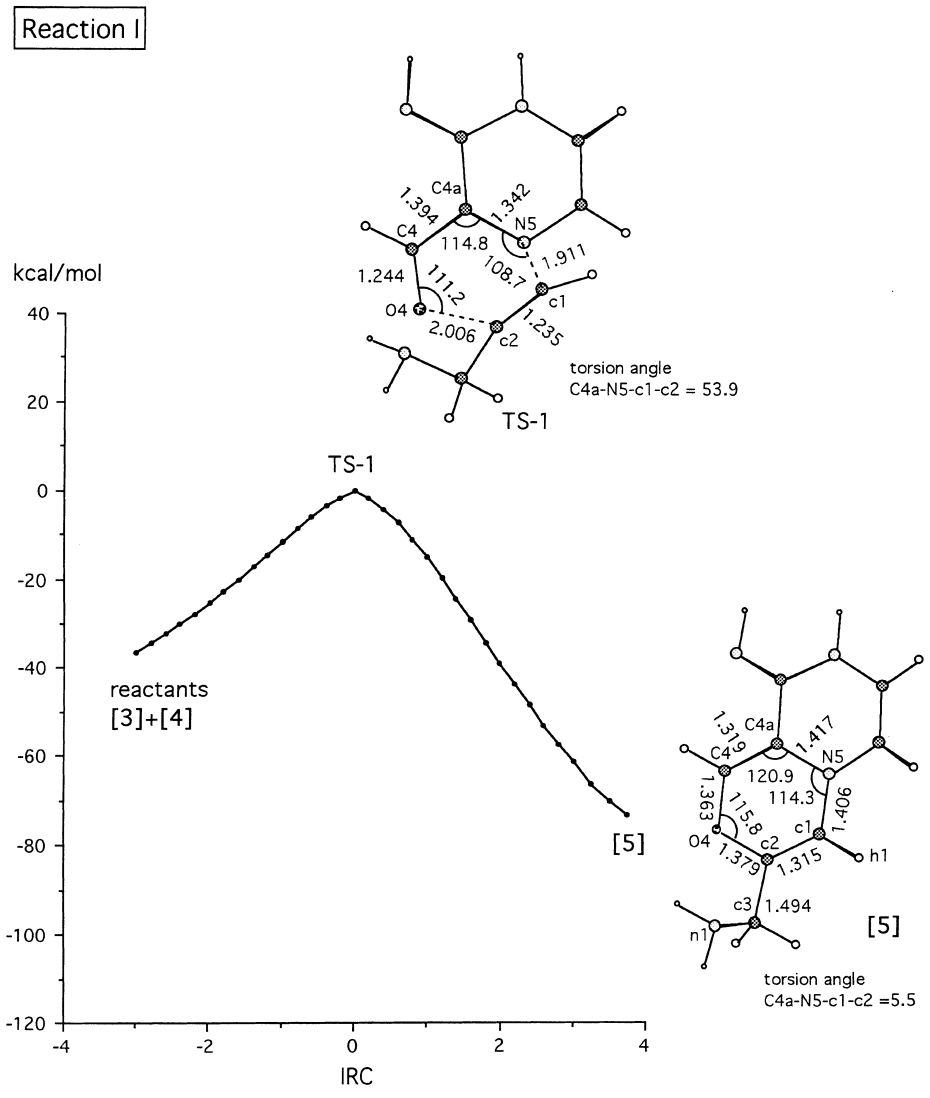


Fig. 4. The transition state and the product in *Reaction* I. The *numbers* are the bond lengths (Å) and bond angles (degree). The relative Hartree-Fock (HF) energy (kcal/mol) curve is presented along the intrinsic reaction coordinate (IRC) in $(amu)^{1/2}$ bohr

considered between atoms C4a and N5 of (3) and atoms c1 and h2 of (4) in which atom c3 comes into the contact through atom h2 as a consequence. Thus a second type of transition state, TS-2, and the product (7) have been found. (7) is an acyclic allenic adduct on C4a of (3) and its total energy is 9.93 kcal/mol lower than the reactants (3) and (4). Figure 5 represents the energy variations along the IRC and the conformations concerned. The activation energy for TS-2 from the reactants is 61.36 kcal/mol which is not much different from that in reaction I. In Fig. 5 another transition state, TS-2′, and the product (7)′, the allenic adduct on N5 of (3), obtained with (4) facing inversely to N5 and C4a of (3) are also represented. As the total energy of (7)′, is just 1.12 kcal/mol lower than that of (7) and the transition state TS-2′ is 7.87 kcal/mol higher than TS-2, it can be considered that these reactions might occur almost equally, except that the imaginary frequency of TS-2′ is 760 cm^{-1} compared to 1989 cm^{-1} for TS-2. This difference can be seen in Fig. 5 as the gradients of the energy curves along the IRC, even though the reaction coordinates are independent of each other. Particularly in the reverse directions toward the reactants from the transition states, the state-like "quasitransition" from TS-2′ is found to continue for a while, and is not seen in the case from TS-2 (see Fig. 5). This implies that the bonding conditions on N5 and on C4a of (3) to c1 of (4) are different. Here TS-2′ and (7)′ seem to be more difficult to make for steric reasons than TS-2 and (7), and the bulky benzene part of (2) omitted in the simplified model (4), may add further difficulty.

4.3 Reaction III: hydrogen migration to another acyclic adduct

The acyclic adduct (7) or (7)′ is only about 10 kcal/mol lower in energy than the reactants. Another type of acyclic adduct, (8)′, has been observed in photochemical reactions [8, 9c, 14, 26, 27]. We have found that these acyclic adducts were brought about by a reaction passing through the transition state TS-3 or TS-3′. This type of mechanism has been suggested previously [10, 12], but was obscure at that time. Our results elucidate it clearly.

The reaction leading through transition state TS-3 or TS-3′ from (7) or (7)′ (Fig. 6) is a hydrogen-transfer one.

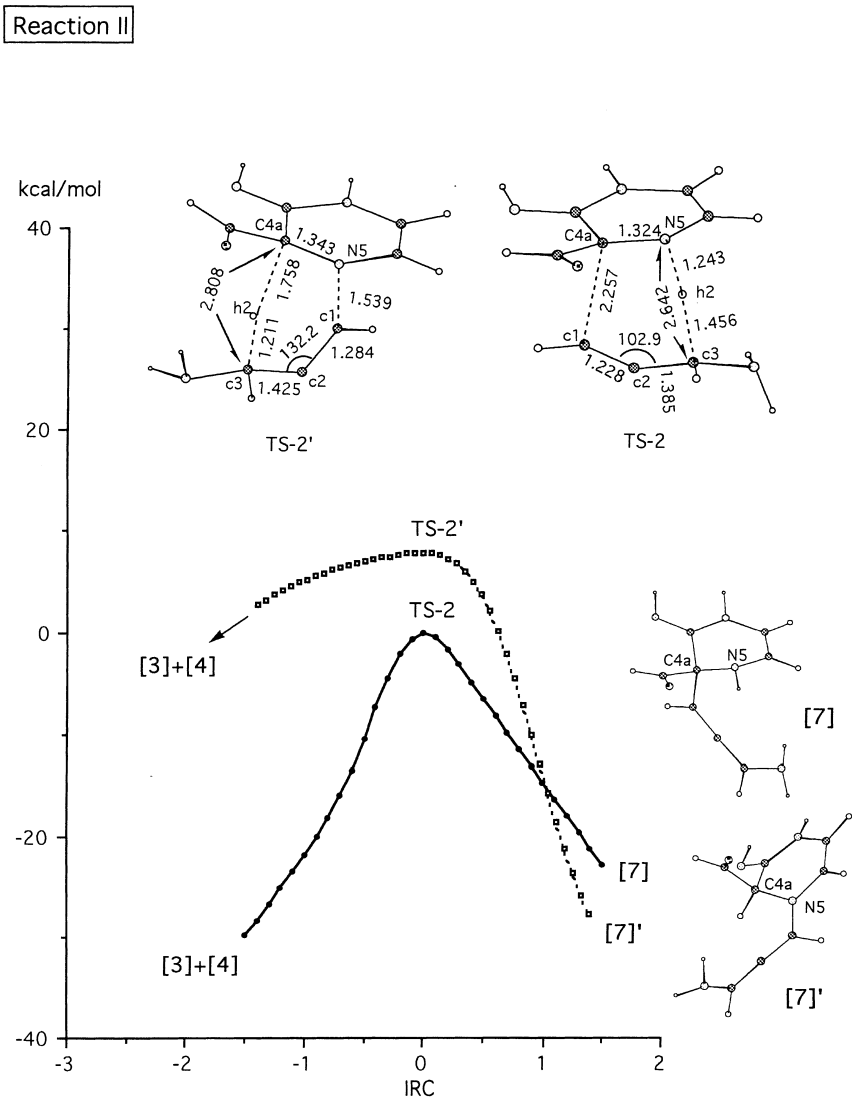


Fig. 5. The transition states and products in *reaction* II. The *labels without* and *with prime* concern with the adducts on *C4a* and *N5*, respectively. The energy curves are independent of each other but are shown conventionally on the same reaction coordinates. Here the two chemical processes, the covalent bond formation and hydrogen transfer take place simultaneously

After reaction II, the hydrogen h2 on N5 of (7) or C4a of (7)′ moves back toward the c2 of the allenic side. The reaction processes (7) → TS-3 → (8) and (7)′ → TS-3′ → (8)′ give rise to the C4a-adduct (8) and the N5-adduct (8)′, respectively, and are shown in Fig. 6. It is observed that their energy curves along the IRC are different particularly in the forward direction and that the equilibrium total energies of (8) and (8)′ are considerably apart from each other. In system B, the adducts on the flavin ring, corresponding to (8) and (8)′ in system A, are called "flavocyanines".

Figure 7 shows comparative pictures of the products (7), (8), (7)′ and (8)′, including geometrical values and whole charges of the inhibitor sides, which we defined as ρ^{I}, of the adducts. (7) and (7)′ have similar bonding features and charge distributions, except for the charges on c1, c2 and h2. Thus both (7) and (7)′ have almost the same total energies, corresponding well to the fact that the difference in their total energies was only 1.12 kcal/mol as found in reaction II. On the other hand, adducts (8) and (8)′ are quite different in geometry and charge distribution. The total energy of (8)′ is 37.57 kcal/mol lower than that of (8). In system B this difference becomes greater and is 61.5 kcal/mol. This is certainly the reason why the N5-type adduct (8)′ has been found in the literature but not the C4a-type one (8).

In the literature [11, 14], the net charge on n1 of the cyanine-type adduct (flavocyanine) has been presented as positive, but our results for (8) and (8)′ have shown negative values of about −0.8 or −0.9 in both systems A and B. Instead, it is noticed that the ρ^{I}s of (8) and (8)′ are positive, 0.8 and 0.6, respectively, and in system B they are both 0.8. Thus the inhibitor sides of (8) and (8)′ are almost imine cation radicals and the flavin model sides of (8) and (8)′ are like anion radicals. On the other hand, the ρ^{I}s of the allenic adducts (7) and (7)′ are 0.04 and 0.2, respectively, and in system B these are 0.2 and 0.4, respectively. The inhibitor sides of adducts (7) and (7)′ are considered to be neutral imine radicals and the flavin model sides of (7) and (7)′ are like neutral semiquinon radicals. These are actually driven through the migration of the hydrogen, h2, which has a charge of about 0.3 to 0.4 (Fig. 7).

Thus, although the reactions discussed here take place between molecules in neutral singlet states, photochemical experimental results of the one-electron

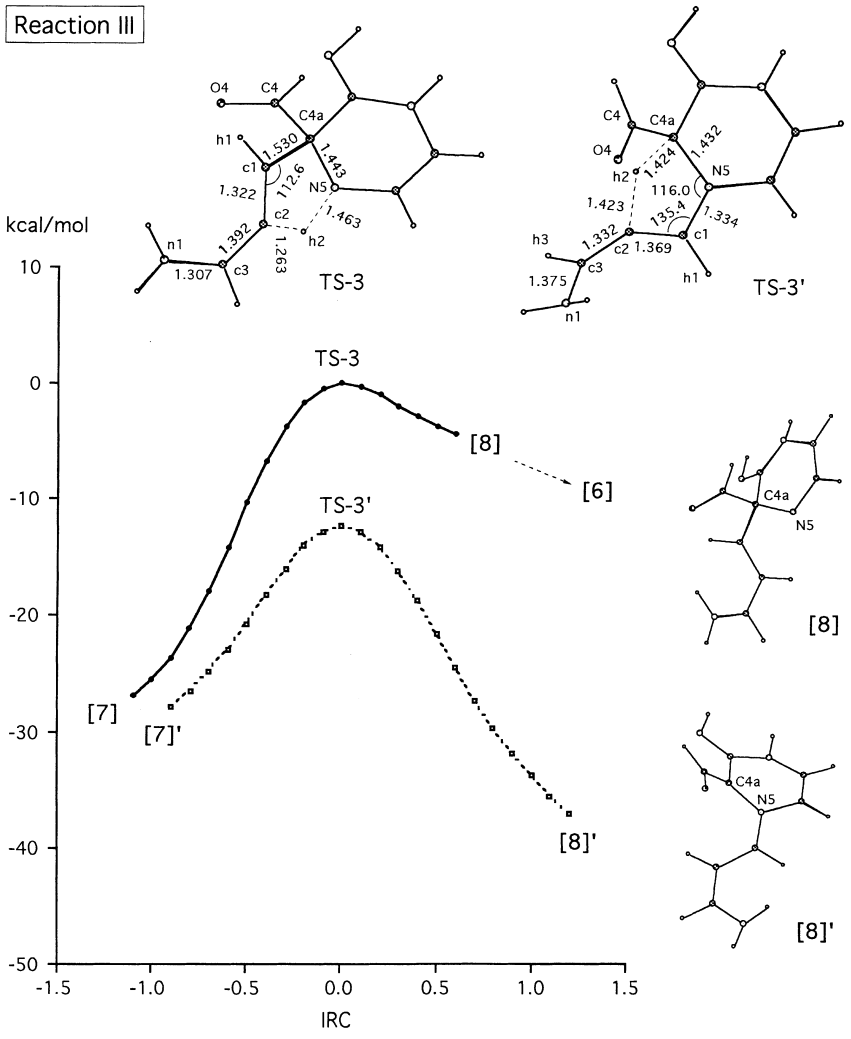


Fig. 6. The transition states and the products in *reaction* III

mechanism could be deduced from the above consideration for the charge distributions.

4.4 Reaction IV: cyclization from (8) *to* (6) *or from* (8)′ *to* (6)′

The frontier orbitals of adducts (8) and (8)′ are shown in Fig. 8. As the conformation of (8) or (8)′ can be turned around the carbon chain, c1-c2-c3, it is considered that cyclization by the interaction between the HOMO on N5 or C4a and the LUMO on c3 is possible when rotation, for which there is almost on energy barrier, results in favorable orbital overlap.

Thus TS-4′ was found to be the transition state for the cyclization from (8)′ to (6)′ which is represented in Fig. 9 together with the energy curve along the IRC. TS-4′ has a large distance (2.71 Å) between the connecting atoms C4a and c3, which indicates that in shorter distance, (8)′ cyclizes spontaneously to (6)′. The variation of this distance along the IRC is also shown on the graph for the energy curve (Fig. 9).

On the other hand, there seems to be no transition state for the cyclization from (8) to (6). (8) is 25.82 kcal/mol higher in total energy than the original reactants (3) and (4) and about 15 kcal/mol higher still than the transition state, TS-4′, from (8)′ to (6)′. It is concluded that (8) goes directly toward (6) without having the transition state, once (8) rotates into a favorable orientation for cyclization. The energy difference between (8) and (6) is 65.30 kcal/mol which is large enough to make spontaneous cyclization possible from unstable (8) to stable product (6).

The geometries and some geometrical values of products (6) and (6)′ are presented in Fig. 9. Products (6) and (6)′ are 39.53 and 35.59 kcal/mol lower in total energy than the reactants (3) and (4), respectively. As (6) and (6)′ are the most stable products obtained, these are considered the final products for inhibition of the flavin function.

(6) and (6)′ have the newly constructed ring consisting of atoms C4a, N5, c1, c2 and c3, which are nearly perpendicular to (3) corresponding to the flavin ring. In (6), the torsion angles of C4-C4a-N5-c3 are 113.2 and 90.8° for systems A and B, respectively. In system B the new ring is more vertical to the flavin ring than in system A. In (6)′, as long as we take model (4) without including the benzene part of (2), these features are almost the same as the ones of (6). The energy differences are only 3.94 and 5.6 kcal/mol for systems A and B, respectively.

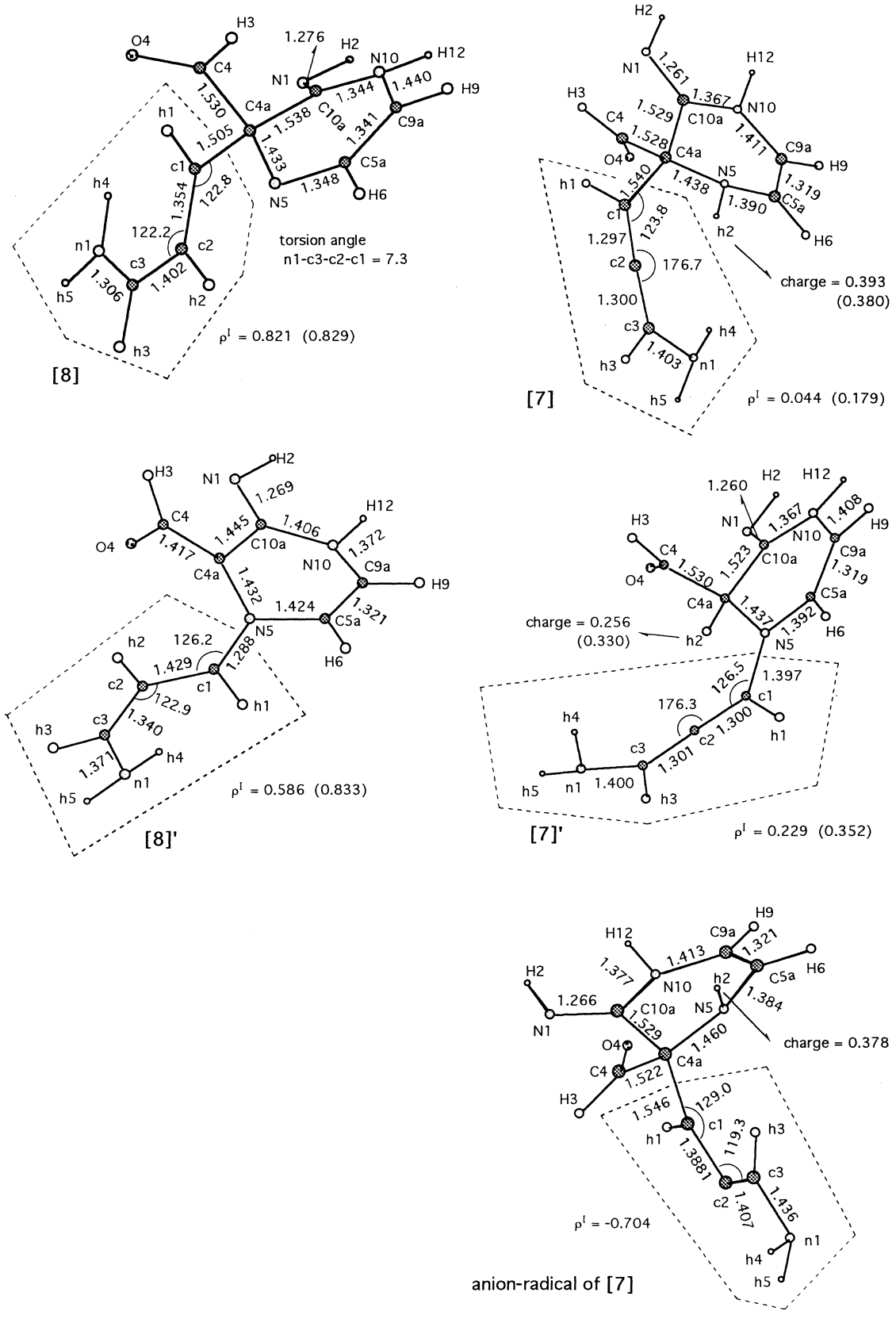


Fig. 7. Comparison of (*7*), (*7*)′, (*8*), (*8*)′ and the anion radical of (*7*). ρ^I is the whole charge (electron unit) of the inhibitor side surrounded by the *dotted lines*. The numbers in *parentheses* are for system B

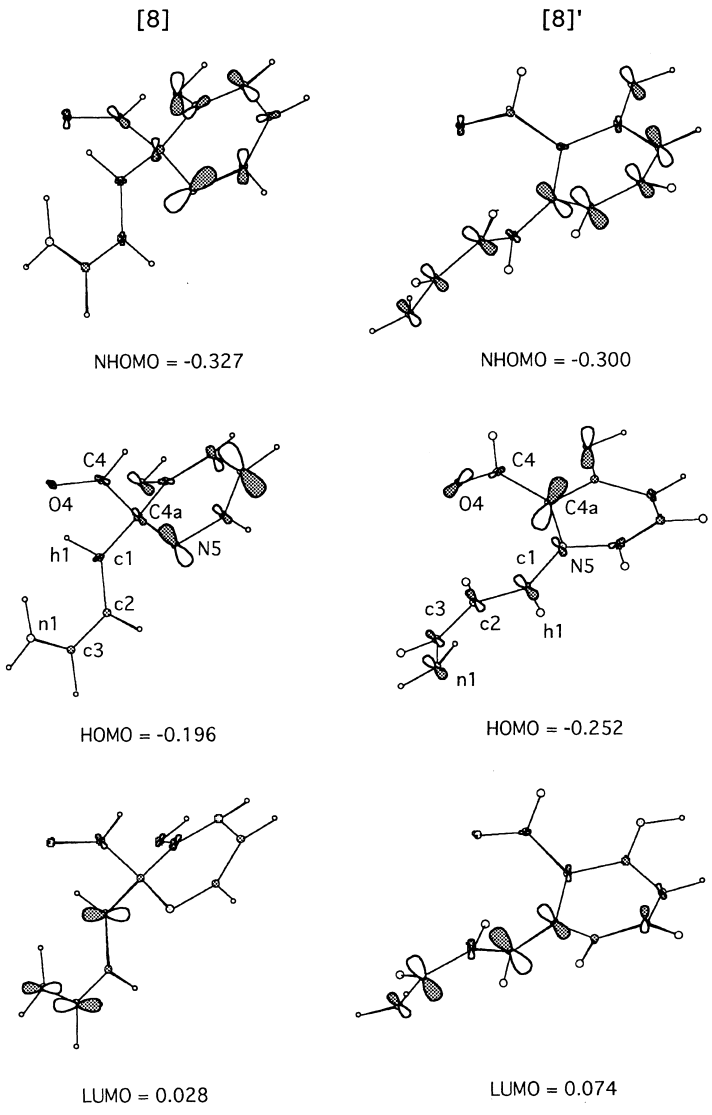


Fig. 8. The MOs of (*8*) and (*8*)′. The energy levels are in atomic units

However, it must be noted that in conformation (6)′ the bond lengths between C4a and c3 are 1.582 and 1.620 Å for system A and B, respectively and that these bonds are very weak or questionable. Also it is reported experimentally that (6) rather than (6)′ was detected [19] in the inhibitions using a different type of inhibitor having phenyl and amino substituents appended to c3.

4.5 Reaction V: another transition state and a hypothetical route

A totally different transition state, TS-5, was involved in the cyclization from (7) to (6). The geometry of TS-5, which has already made a ring conformation with atoms N5, C4a, c1, c2 and c3, and atom h2 situated just on the upper middle of this new ring, is shown in Fig. 10.

The product in the reverse direction from TS-5, from which the activation energy to TS-5 is 99.33 kcal/mol too high, is (7). The energy curve along the IRC indicated the existence of an intermediate state between (7) and TS-5, and one equilibrium state, (9), which was very unstable, has been found. (9) was found only with the STO-3G basis set, and this was also the case for the corresponding intermediate in system B. The energies and the molecular orbitals of (9) were calculated using the 6-31G* and the 3-21G basis sets in systems A and B, respectively, for the conformations found with the STO-3G basis set. (9) converts easily into (7) or TS-5 with neither a barrier toward (7) nor toward TS-5. The total energy of (9) is 54.37 kcal/mol higher than that of (7) and 44.94 kcal/mol lower than that of TS-5.

Compound (10) was obtained in the forward direction from the transition state TS-5 (Fig. 10). This was a very unstable singlet carbene-type product and was 23.25 kcal/mol higher in total energy than the original reactants [3] and [4]. The final product (6) was easily obtained when one more transition state TS-6, to which the activation energy from (10) was only 17.01 kcal/mol, was passed. This reaction is a 1,2-hydrogen-transfer reaction [28]. The hydrogen h2 sits vertical to the appended plane consisting of atoms N5, C4a, c1, c2 and c3. The existence of the carbene-type intermediate (10) is

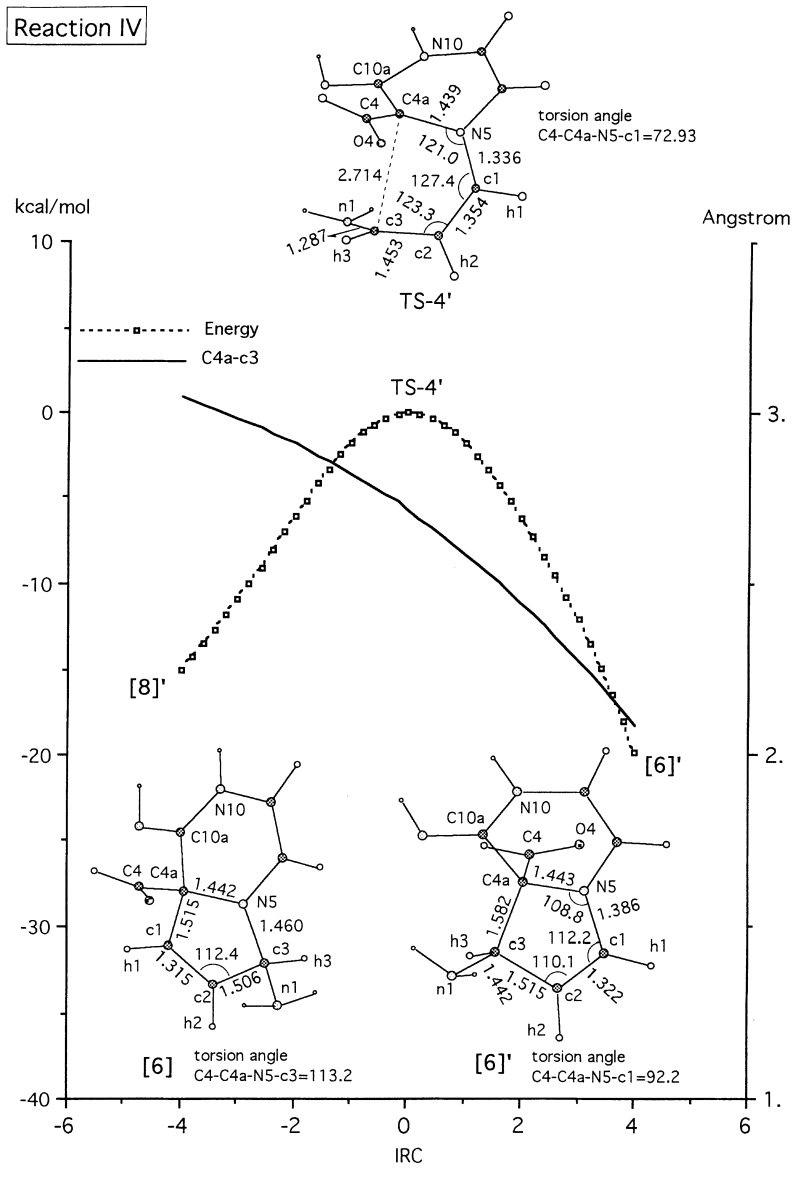


Fig. 9. The transition state and products in *reaction IV*. The variations of the distance C4a-c3 (Å) and of the relative HF energy along the IRC as shown

interesting, because the carbene intermediate has recently been reported to be involved in the activity of orotidine monophosphate decarboxylase [29].

Even with the existence of intermediate (9) and the final product easily obtained from TS-5, the high energy barrier from starting product (7) makes this route impossible to follow and the conformationally very straight allenic angle of c1-c2-c3 of (7), 176.7° (Fig. 7), is maybe the bottleneck. Figure 11 represents the frontier orbitals of the molecules involved in the reaction routes from (7) to TS-5, in which the molecular orbitals of the triplet and anion radical states of (7) are included for comparison. The triplet and the anion-radical states of (7) have the bent forms of the angle c1-c2-c3 which are 143.66 and 119.30°, respectively, and their total energies are 25.48 and 42.81 kcal/mol higher than that of the singlet state, respectively.

In Fig. 11, the phases of the frontier orbitals of the triplet state of (7) show clearly that one electron in the HOMO in the singlet state of (7) is excited into its LUMO. On the other hand, in the anion-radical state of (7), the exchange of the energy levels of the molecular orbitals takes place and it is observed that the phase of the SOMO of the anion-radical state (7), which corresponds to the NHOMO of the singlet state of (7), is similar to the HOMO of (9). The phases of the HOMOs of (9) and TS-5 are very similar to each other and there is no level exchange of the molecular orbitals when (9) goes to TS-5. The frontier orbitals of the anion-radical state of (7) are already switched to ones type (9) or TS-5 but the ones of the triplet state of (7) are not. Thus together with the fact that the energy difference between the anion-radical state of (7) and (9) is only 12 kcal/mol, it would be interesting to make the hypothesis that an anion-radical state intervenes in the reaction processes; for example, in the middle of the path from TS-2, the system gets one electron from somewhere before falling down to the singlet state of (7) and converts into the anion-radical state which will make a short-cut to (9) while losing the electron again.

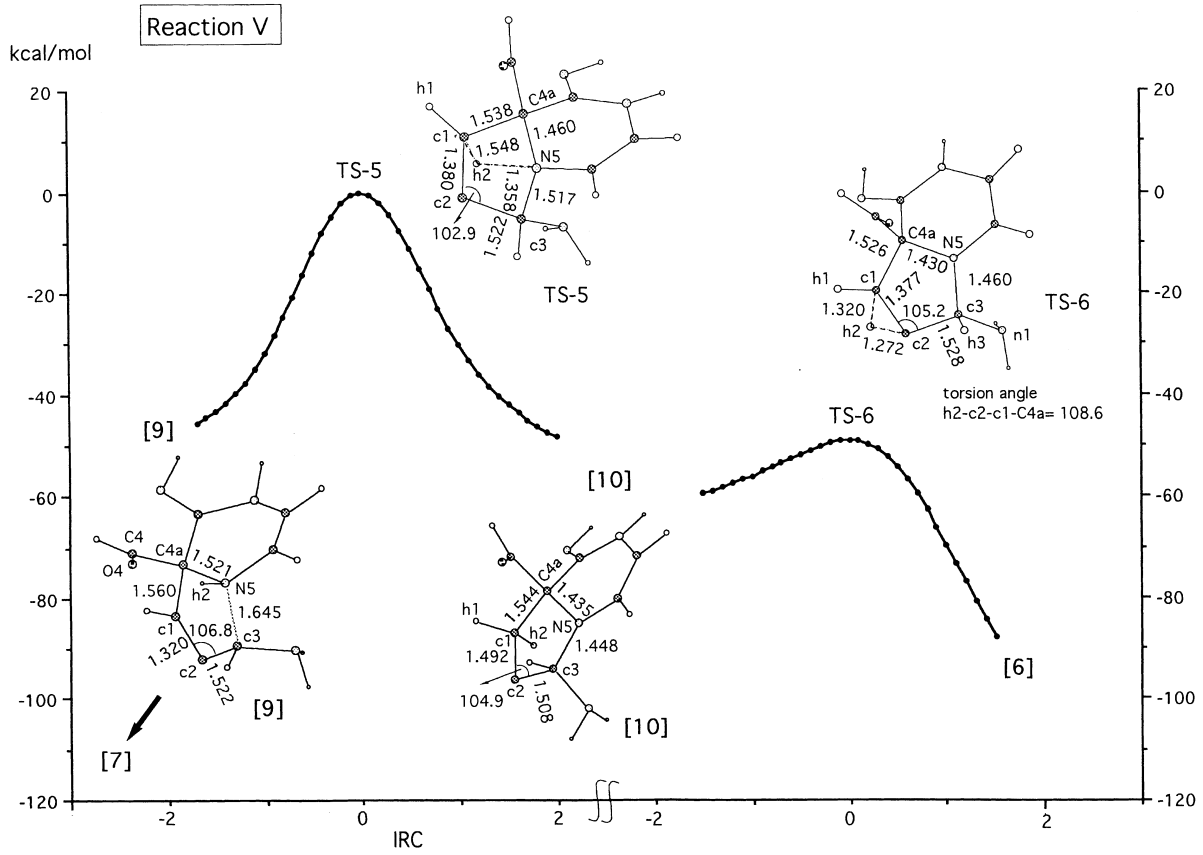


Fig. 10. The reactants, transition states and products in *reaction V*. Two reactions passing through transition states *TS-5* and *TS-6* are shown. The HF energies are relative to *TS-5* for both energy curves

5 Energy diagram and frequency analysis

The energy diagrams for all the states mentioned above are shown in Fig. 12. The scaling used is for the energy values of system A. The numbers in parentheses are the corresponding energy values of system B which are not scaled.

Figure 13 represents the imaginary vibrational modes of all the transition states found in this study. Except for TS-1 and TS-4′, it is interesting to note that hydrogen h2 moves mainly in these transition states against a background of almost immobile atoms.

6 Discussion

In reaction II, the existence of transition states TS-2 and TS-2′ is of considerable interest, because two chemical processes take place simultaneously: covalent bond formation and hydrogen transfer between (3) and (4) to give the acyclic allenic adducts (7) and (7)′. However, the hesitation in the reverse direction from TS-2′ might imply less simultaneity of the above two processes in TS-2′ than in TS-2. Although the activation energies for reactions I and II are quite high, about 60 kcal/mol, these values are reduced by about 14 kcal/mol in system B certainly due to the effect of the tricyclic flavin ring (see the energy diagrams, Fig. 12), and in a real enzymatic system they would be reduced much more.

In the meantime, (7) and (7)′ could arise from enzymatic oxidation of (4) to the oxidized imine radical, which attacks the reduced hydro-(3) radical corresponding to the flavin semiquinon radical. Alternatively, the formation of (8) and (8)′ could be possible by combination of the radical anion of (3) and the radical cation of (4). Actually Silverman has suggested the intervention of the flavin radical anion in a single-electron mechanism. Although we have not pursued these reactions, we have found in the reactions studied here that hydrogen migration plays an important role causing switching between the oxidized, radical and reduced states of the inhibitor side and the flavin model side. Our results clarify analytically what essentially happens between the acetylenic inhibitor and flavin.

As long as we take the experimental results into consideration, the routes of reactions III and IV from products (7) or (7)′ toward the final product (6) are reasonable. On the other hand, the route of reaction V is interesting for the reaction of the flavin ring but is unlikely because of the high energy barrier from (7) to TS-5. Although we considered a hypothetical route concerning with the anion-radical state, we did not find any proof for it.

In the first step of this study we got a singlet state quadrangular adduct, C4a-N5-c2-c1, whose total energy is 9.25 kcal/mol lower than that of the reactants [3] and [4]. The quadrangular plane was almost vertical to the ring plane. The phases of the SOMO of the triplet-state of (1) or (3) at C4a and N5, which correspond to

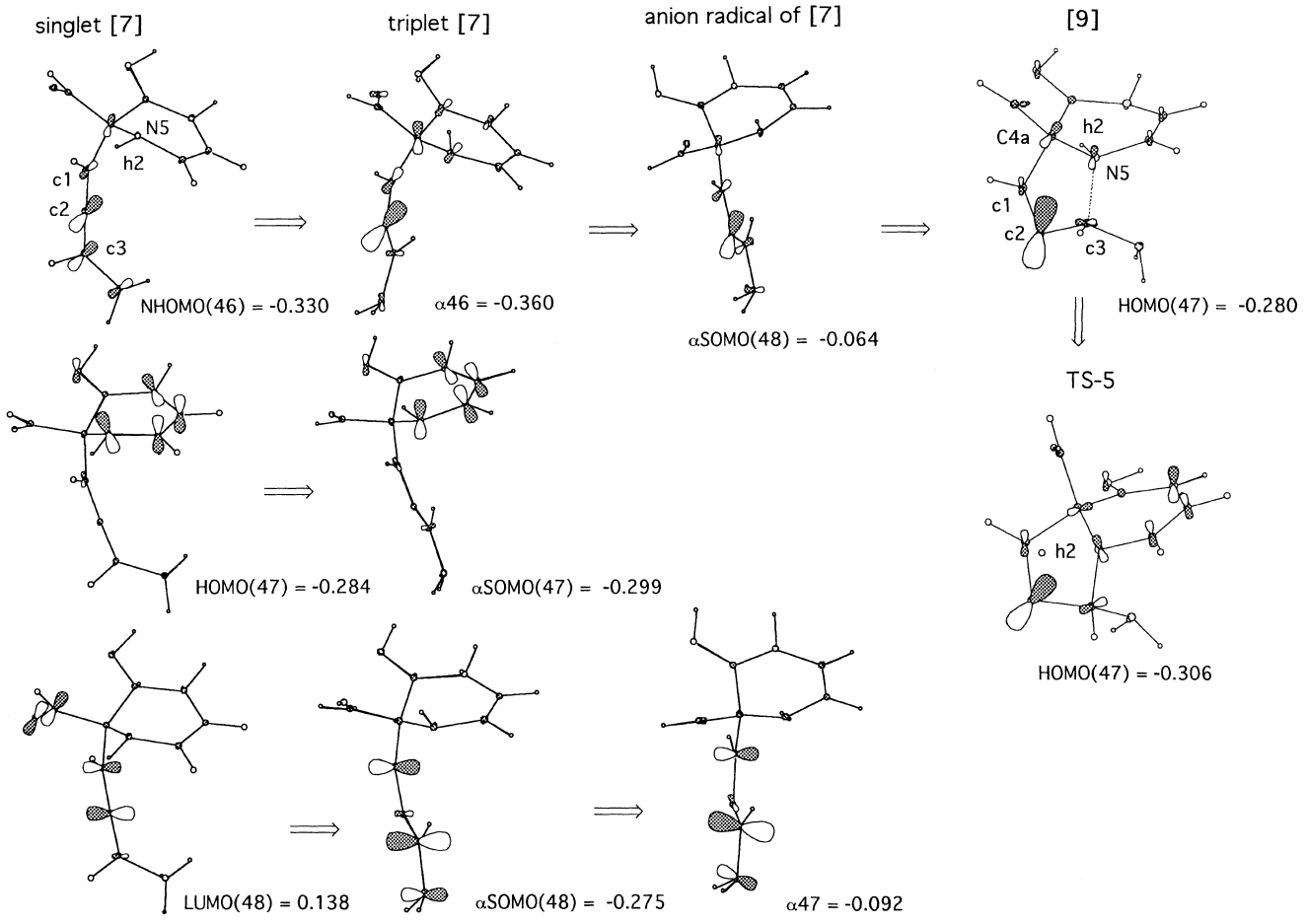
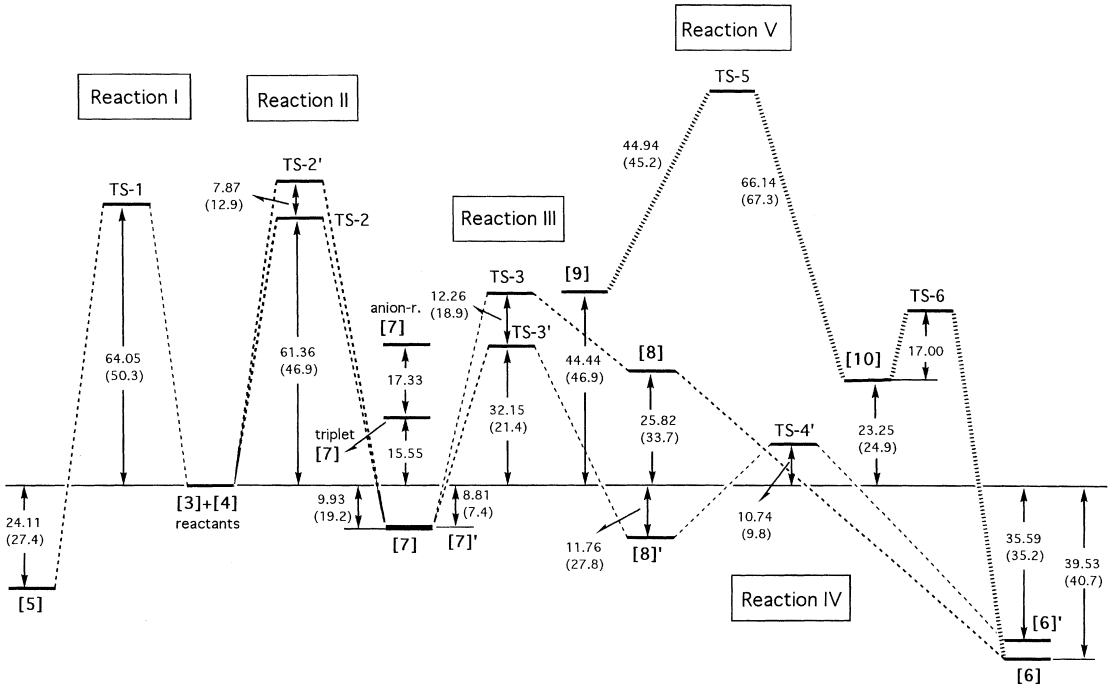


Fig. 11. The phase relations among the MOs of (*7*), the triplet of (*7*), the anion radical of (*7*), (*9*) and *TS-5*. The MOs with similar phases are connected by *arrows*



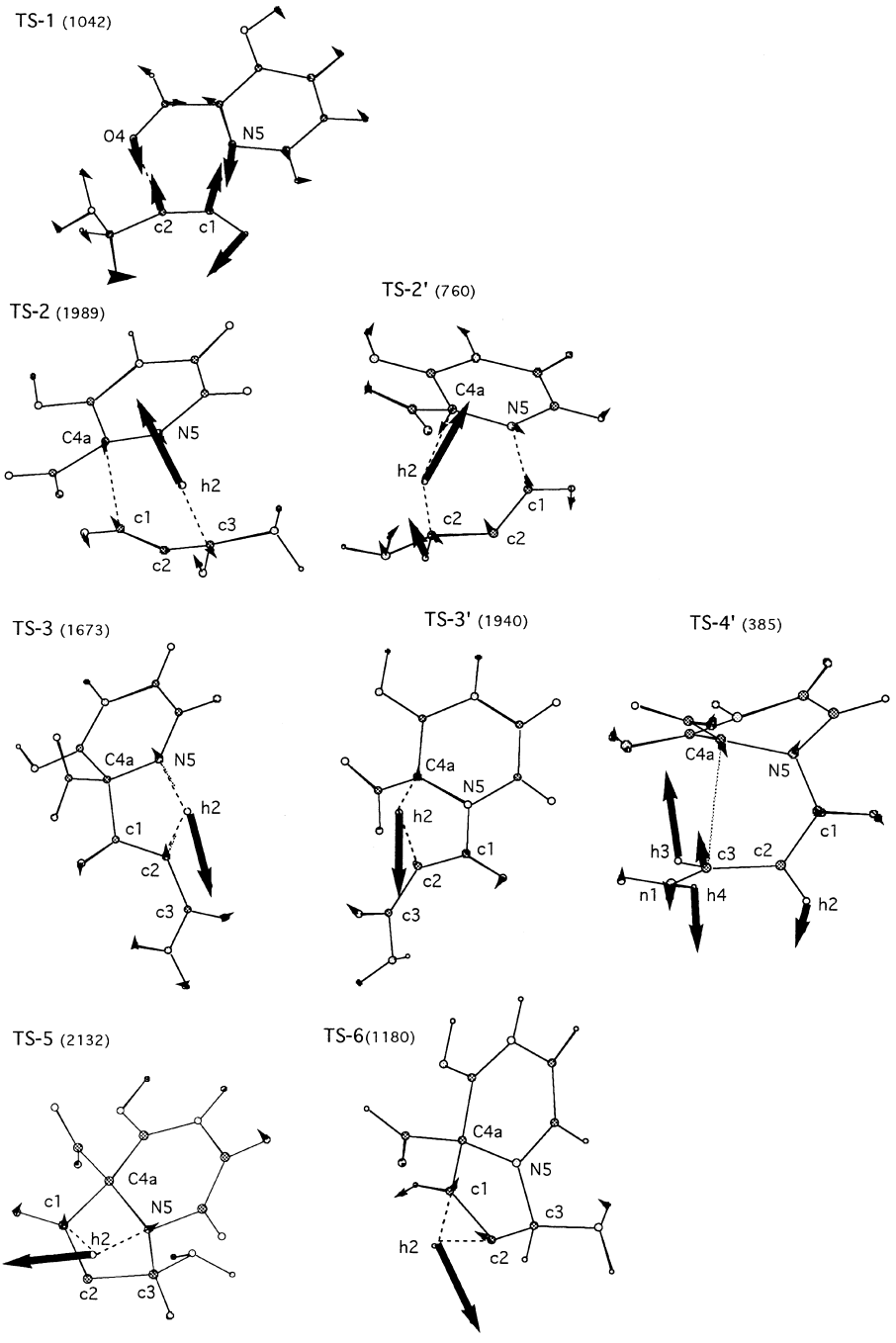


Fig. 13. The imaginary frequency modes (cm^{-1}) of all the transition states

the phases of the LUMO of the singlet state, are favorable to those of the LUMO of the acetylenic part, c1 and c2 of (4) (Fig. 3). These are concerned with the forbidden supra–supra reaction which should take place through a thermal two step-reaction or through a photochemical reaction [30]. Although it was possible to find the transition state and the product in the thermal two-step reaction leading to the quadrangle using the smaller models consisting of atoms C4a, N5, c1, c2 and hydrogens, it was not possible with models (3) and (4) because of the intervention of atoms c3, n1 and O4 in the reaction. It was not possible to find this quadrangular product in the one-step reaction with the lowest triplet states of models (3) and (4), in consideration of the permitted photochemical reaction, by means of the usual UHF method with the 6-31G* basis set.

◀

Fig. 12. Energy diagram of all reactants, transition states and products. Energies are in kcal/mol. The HF energies are relative to the initial reactants (*3*) + (*4*), for which the total energy is −601.2898 hartrees from the calculation with the 6-31G* basis set. The HF energies (in *parentheses*) of system B are relative to the initial reactants (1) + (4), for which the total energy is −915.4682 hartrees from the calculation with the 3-21G basis set

7 Conclusion

The inhibition mechanisms of MAO-B by (2) have been studied theoretically using the simplified models (3) and (4). According to frontier orbital theory, two cyclic products, Diels-Alder type O4,N5-cyclic adduct (5) and C4a,N5-cyclic adduct (6), have been found. (5) was the product formed by a one-step Diels-Alder cycloaddition reaction. (6) was most stable product in which the new ring is perpendicular to (3) corresponding to the flavin ring plane, and is formed without having gone through intermediates or transition states. As intermediates, the acyclic C4a- and N5-allenic compounds, (7) and (7)′, and their hydrogen-transferred cyanine-type compounds, (8) and (8)′, have been found. N5-adduct (8)′ is the same type as the product obtained in experimental photochemical reactions. This is reasonable since (8)′ is much more stable than (8). Instead (8) goes forward much more easily than (8)′ in the next reaction which is the cyclization toward the final C4a,N5-cyclic adduct (6). (6) can be obtained without an energy barrier from (8), while there is an energy barrier from (8)′ to (6)′. Thus all these results indicate that only the route toward (6) occurs. This is again compatible with the fact that type (6) rather than (6)′ has been detected in experiments.

In another reaction route from (7) to (6), intermediate (9) and then a carbene-type intermediate (10), which was very unstable and progressed easily toward the final product (6), have been found. Lastly it is remarked that in most of the processes, hydrogen migration plays an important role in driving the reactions.

As the MAOs are related to the regulations of not only dopamine but also other neurologically active amines in the brain, the study presented here may be helpful in the design of new MAO inhibitors.

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