Biophysical Chemistry 12 (1980) 13–19

QUANTUM-CHEMICAL STUDY OF CHYMOTRYPSIN PRIMARY SPECIFICITY

Pavol BAŇACKÝ
Scientific-Research Institute, Faculty of Pharmacy, Comenius University, 880 34 Bratislava, Czechoslovakia

Received 6 August 1979
Revised manuscript received 3 January 1980

It is assumed that the primary specificity of chymotrypsin is determined by the formation of a molecular complex between the aromatic nucleus of the side chain of the specific substrate cleaved bond and the peptide bond of chymotrypsin "tosyl hole". The suggested model has been studied by means of the quantum chemical method, CNDO/2 and perturbation theory up to second order. The obtained results show the possibility of formation of molecular complexes of the above mentioned type.

1. Introduction

Chymotrypsin primarily catalyzes the hydrolysis of peptide bonds of proteins adjacent to the carbonyl of the aromatic amino acids. The enzyme also catalyzes the hydrolysis of low molecular amides and esters of aromatic amino acids. In comparison with catalytic efficiency of the enzyme towards hydrolysis of peptide bonds formed by tryptophan (Trp), thyrosine (Tyr) and phenylalanine (Phe) (100%, 91% and 83%), its efficiency for hydrolysis of peptide bonds formed by histidine (His) is surprisingly low (31%) [1].

It is obvious that the specificity of every enzyme is directly related with the character of the enzyme–substrate (ES) complex of corresponding enzymatic process. The problem of chymotrypsin primary specificity is, therefore, reduced to the clarification of the interaction between the side chain of the specific substrate (essentially the particular aromatic nucleus) and the enzyme sorption region.

UV spectra of cinnamoylchymotrypsin [7,8] contradict the widely accepted notion about the hydrophobic nature of the interaction at the formation of ES complex for the chymotrypsin catalyzed processes [2–6]. Analysis of these spectra showed that the benzene nucleus of cinnamoyl is placed in the enzyme sorption region of high polarity. Results of X-ray diffraction of chymotrypsin, its acyl derivates and its complexes with inhibitors show that the enzyme has only one sorption region for the side chain of specific substrates – the so called "tosyl hole" [9–13]. Sorption region which due to its shape looks like a "pocket" at the surface of the enzyme, consists of $-CH_2$-groups of different aminoacid residues whereas the peptide bonds Trp 215–Gly 216 and Cys 191–Met 192, are situated on the opposite sides of the "pocket" in parallel planes. These peptide bonds are not solvated at the time of ES complex formation and the aromatic nucleus of the specific substrate is placed between them in a plane parallel to those of the said bonds [14,15].

On the basis of the geometrical arrangement (X-ray data) and the character of the interacting partners, it may be assumed that the primary specificity of chymotrypsin is determined by the formation of molecular complex between the aromatic nucleus of the specific substrate side chain and the peptide bond of the "tosyl hole" enzyme.

Theoretical study of the formation of such molecular complexes is the subject of the present work.

2. Methods and studied models

In our case it is possible to take into consideration the interaction of the aromatic nucleus with the isolated peptide bond as the model which retains the basic characteristics of the ES complex. From among the aromatic nuclei, indol (Trp), phenol (Tyr) and benzene

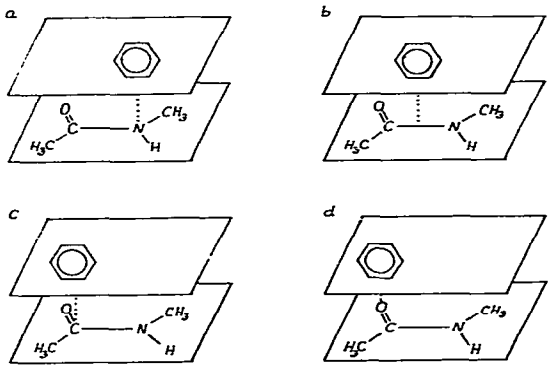


Fig. 1. Studied models of molecular complex formation between benzene and N-methylacetamide.

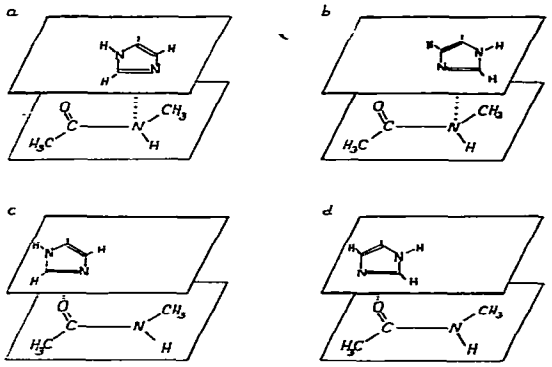


Fig. 2. Studied models of molecular complex formation between imidazole and N-methylacetamide.

(Phe), the benzene molecule was selected for the study on the basis of the assumption that the non polar benzene molecule would form the least stable complex with the polar peptide bond. In an attempt to explain the low chymotrypsin specificity towards hydrolysis of "histidine" peptide bonds, the interaction of peptide bond with the imidazole (His) was also studied. In both the cases the planar molecule of trans-N-methylacetamide (NMAA) was used as the model of peptide bond "tosyl hole" enzyme. The formation of the molecular complex between benzene and NMAA was studied for four different planparallel arrangements (fig. 1). The geometrical arrangements of the molecules for the interaction of NMAA with imidazole are shown in fig. 2.

The above introduced model systems were studied by two different quantum chemical methods:

a) The standard version of CNDO/2 method [16]. The interaction energy ΔE_{AB} was in this case calculated by the method of "supermolecule".

$$\Delta E_{AB}(r) = E_{AB}(r) - (E_A + E_B)\,. \qquad (1)$$

b) The many-body perturbation theory up to second order [17] in approximation of CNDO/2 hamiltonian [18]. For the total interaction energy E_{AB} of two closed-shell molecules A and B in ground electronic states holds the relation — eq. (2).

$$\begin{aligned}
E_{AB}(r) = {} & \sum_{a}^{A}\sum_{b}^{B} [(Q_aQ_b - Q_aZ_b - Q_bZ_a)\gamma_{ab} + Z_aZ_bR_{ab}^{-1}] \\
& + \sum_{i\in A}^{un}\sum_{j\in A}^{occ} 2(\epsilon_j - \epsilon_i)^{-1}\left[\sum_{a}^{A}\sum_{b}^{B}\sum_{\mu}^{a} c_{i\mu}c_{j\mu}\Delta Q_b\gamma_{ab}\right]^2 \\
& + \sum_{i\in B}^{un}\sum_{j\in B}^{occ} 2(\epsilon_j - \epsilon_i)^{-1}\left[\sum_{a}^{A}\sum_{b}^{B}\sum_{\nu}^{b} c_{i\nu}c_{j\nu}\Delta Q_a\gamma_{ab}\right]^2 \\
& + \sum_{i\in B}^{un}\sum_{j\in A}^{occ} 2(\epsilon_j - \epsilon_i)^{-1}\left[\sum_{\mu}^{A}\sum_{\nu}^{B} c_{j\mu}c_{i\nu}h_{\mu\nu}\right]^2 \\
& + \sum_{i\in A}^{un}\sum_{j\in B}^{occ} 2(\epsilon_j - \epsilon_i)^{-1}\left[\sum_{\mu}^{A}\sum_{\nu}^{B} c_{i\mu}c_{j\nu}h_{\mu\nu}\right]^2 \\
& + \sum_{i\in B}^{occ}\sum_{l\in A}^{occ}\sum_{j\in B}^{un}\sum_{k\in A}^{un} 4(\epsilon_i + \epsilon_l - \epsilon_j - \epsilon_k)^{-1} \\
& \times \left[\sum_{a}^{A}\sum_{b}^{B}\sum_{\mu}^{a}\sum_{\nu}^{b} c_{i\nu}c_{j\nu}c_{l\mu}c_{k\mu}\gamma_{ab}\right]^2, \qquad (2)
\end{aligned}$$

where a and b are atoms on the systems A and B respectively, having nuclear charges Z_a and Z_b. Q is the localized charge defined as:

$$Q_a = 2\sum_{\mu}^{A}\sum_{i\in A}^{occ} c_{i\mu}^2\,; \quad Q_b = 2\sum_{\nu}^{b}\sum_{j\in B}^{occ} c_{j\nu}^2\,,$$

and $\Delta Q_i = Z_i - Q_i$.

The electronic repulsion integrals γ_{ab} are calculated

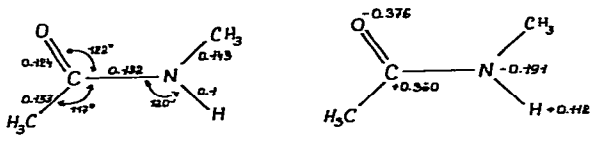


Fig. 3. Calculated geometry and net charge distribution of N-methylacetamide by CNDO/2 method.

theoretically as in CNDO/2 method. The one particle integrals $\check{n}_{\mu\nu} = \langle\mu|\hat{h}|\nu\rangle$ are approximated by the same manner as in CNDO/2 method. In evaluating the various interaction terms in eq. (2), the CNDO/2 wave functions and orbital energies of isolated molecules A and B are used.

The physical interpretation of the individual interaction terms in eq. (2) is as follows:

$$E_{AB}(r) = E_{Coul.} + E_{Pol.}^{(A\leftarrow B)} + E_{Pol.}^{(A\rightarrow B)} + E_{CT}^{(A\rightarrow B)} + E_{CT}^{(A\leftarrow B)} + E_{Disp.} \cdot \qquad (3)$$

The exchange repulsion interaction term is not included in eq. (2) because of ZDO approximation in CNDO/2 hamiltonian.

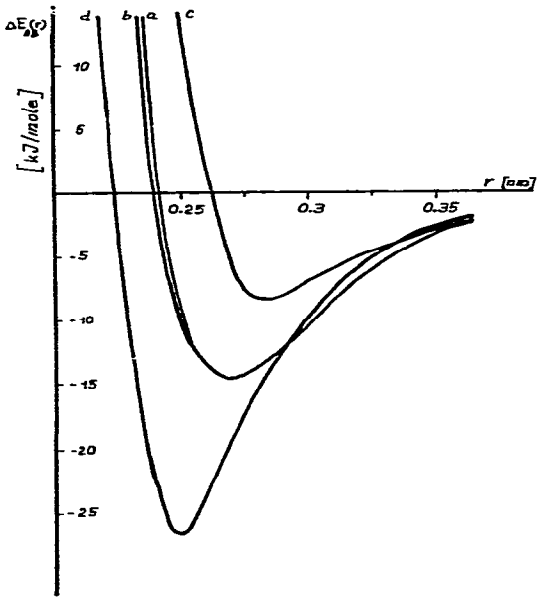


Fig. 4. Interaction energy curves calculated for the system benzene/N-methylacetamide by CNDO/2 method.

3. Results

3.1. The system: NMAA–benzene

The geometry of NMAA molecule obtained by gradual optimization of bond lengths and bond angles by CNDO/2 method was used for the calculation of the interaction – fig. 3. Interatomic overlap populations of C=O and C–N bonds for the NMAA molecule of optimized geometry are:

$$p(C{=}O) = 1.74\,, \quad p(C{-}N) = 1.66\,.$$

The benzene molecule was used for calculation in the standard geometry with bond lengths C–C = 0.1397 and C–H = 0.1084 nm. The interaction energy was calculated along the reaction coordinate connecting the midpoint of the benzene molecule with the respective atom or with the centre of C–N bond of NMAA molecule (fig. 1a–d). The internal coordinates of the interacting molecules were preserved while calculating the interaction energy. Potential energy curves of mutual interaction obtained by CNDO/2 method for the

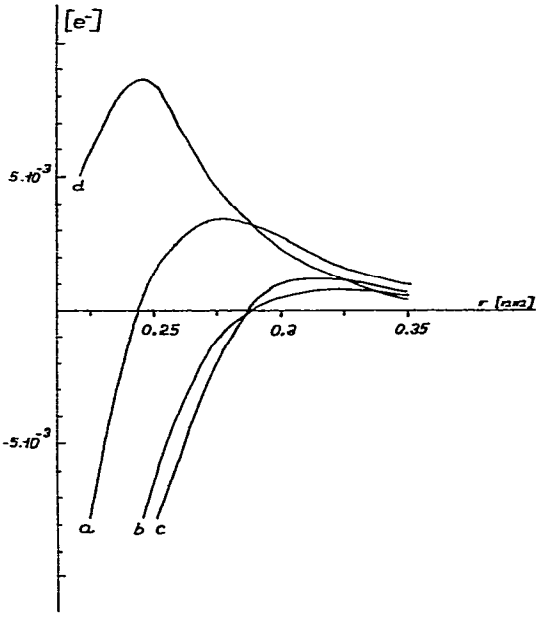


Fig. 5. Dependence of benzene net charge on the distance of interacting molecules. The system benzene/N-methylacetamide.

Table 1
Interaction energy of the system benzene/N-methylacetamide (fig. 1a) calculated by perturbation theory. * total dispersion energy

Distance of molecules [nm]	Energy [kJ/mole]						
	Coulomb.	Polarization		Disp.	Charge-transfer		Total
		A ← B	A → B		occA/unB	occB/unA	
0.25	129.62	0.0	−0.62	−1.66	−48.52	−58.45	20.56
0.275	50.9	0.0	−0.43	−1.19	−21.02	−30.6	−3.01
0.3	19.05	0.0	−0.31	−0.85	−8.66	−15.41	−6.24
				−2.95*			−8.34*
0.325	7.03	0.0	−0.22	−0.60	−3.43	− 7.49	−4.65
0.35	2.56	0.0	−0.16	−0.44	−1.34	−3.51	−2.85

A — N-methylacetamide; B — Benzene

studied geometrical arrangements are shown in fig. 4. Dependence of net charge of benzene molecule on the distance between the interacting molecules for different geometrical arrangements is shown in fig. 5.

Results obtained by using perturbation theory — eq. (2), for the geometrical arrangement of the interacting molecules shown in fig. 1a are presented in table 1. The dispersion energy was calculated only for 5 highest occupied and 3 lowest unoccupied MO's per interacting molecule. The calculation of total dispersion energy was then carried out at equilibrium distance of interacting molecules.

3.2. The system: NMAA—imidazole

The geometry of NMAA molecule was the same as in case of the preceding system. The experimental geometry (X-ray) was used for imidazole molecule [19]. The reaction coordinate is along the line connecting the mid-point of imidazole with nitrogen (fig. 2a,b) or oxygen atom (fig. 2c,d) of NMAA molecule.

The interaction energy was calculated by CNDO/2 method. Its course for different geometrical arrangements is shown in figs. 6 and 7.

4. Discussion

It is known that the CNDO/2 method is quite suitable for the studies of molecular complexes. Molecular complexes of the type $b\pi - a\pi$ [20,21] are, however, exceptions. Satisfactory results were obtained by using this method in the study of $b\pi$—$a\sigma$ and n—$a\sigma$ complexes [22—27].

It is not possible in the case of molecular complexes studied in this work to express "a priori" the type of complexes formed. As it is obvious from the calculated values of interatomic overlap populations for C=O and C—N bonds, the C—N bond in NMAA molecule is partly

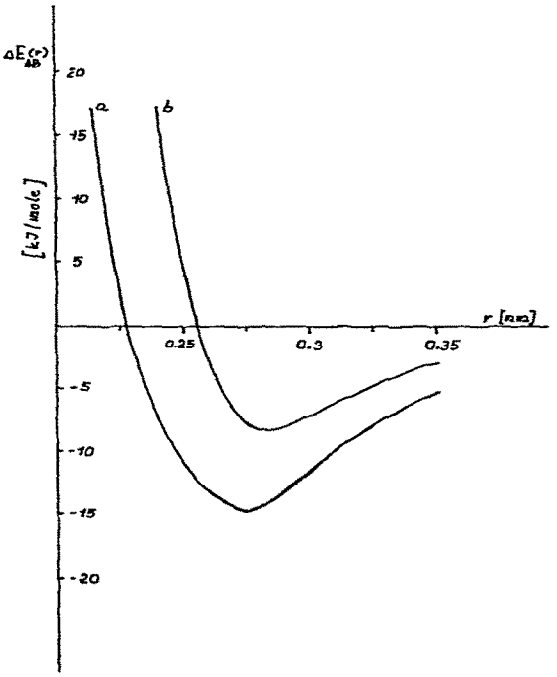


Fig. 6. Interaction energy curves calculated for the system imidazole/N-methylacetamide by CNDO/2 method (geometry fig. 2a,b).

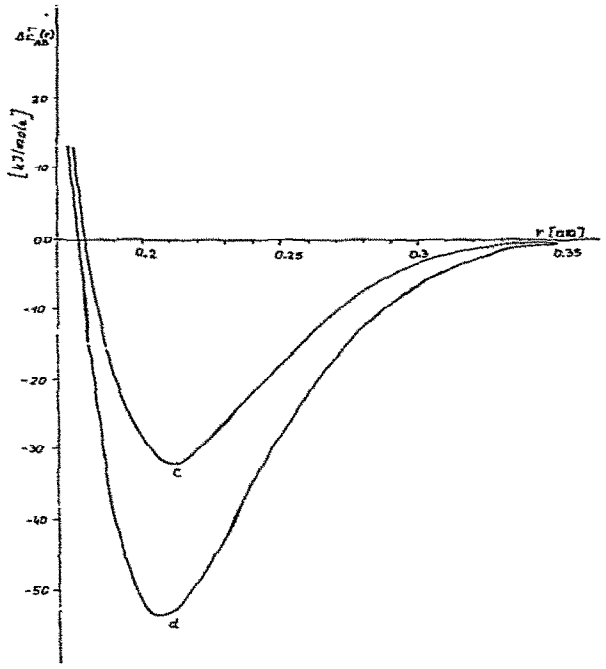


Fig. 7. Interaction energy curves calculated for the system imidazole/N-methylacetamide by CNDO/2 method (geometry fig. 2c,d).

of the double bond character. It may, therefore, be assumed that at the interaction with benzene or imidazole, the molecule of NMAA can act as electron acceptor as well as electron donor (partly as aσ type and n or bπ type). Probably due to this back donation effect the total transferred charge at the interaction of NMAA with benzene is relatively small (fig. 5). Moreover, the donor-acceptor ability of the interacting molecules changes with mutual geometrical arrangement. At equilibrium distances of interacting molecules in geometrical arrangements fig. 1a and d, the NMAA molecule is an electron acceptor – fig. 5a,d. Interaction energies in these cases are −14.65 kJ/mole and −27.2 kJ/mole (fig. 4a,d). In geometrical arrangements fig. 1b,c of interacting molecules at equilibrium distances the NMAA molecule is electron donor – fig. 5b,c. Corresponding interaction energies are −14.65 kJ/mole and −8.37 kJ/mole.

The results obtained by perturbation theory have confirmed the assumption about the back donation effect at the interaction of studied molecules. Table 1 shows the results obtained for the interaction of NMAA with benzene in the geometrical arrangement fig. 1a. It follows from the results that main stabilization contribution towards interaction energy comes from forces of the type charge transfer (CT). At the equilibrium distance of 0.3 nm the back donation energy corresponds roughly to 36% (electron transfer from NMAA to benzene) of the total charge transfer energy. The total dispersion energy represents the second most important stabilization contribution. Contributions due to polarization energy have correct trend but probably their values are very low. The repulsion contribution to the interaction energy is realized through Coulomb forces. This is, however, artefact of the used method. ZDO approximation used in CNDO/2 hamiltonian excludes the exchange repulsion contribution from the expression for the total energy. Consequently, the repulsion forces of short range are then erroneously reproduced through a Coulombic term. One of the reasons for too high repulsion values of the Coulombic term at short intermolecular distances may be the fact that this term in $E_{AB}(r)$ is calculated through charge distribution of isolated molecules A and B. In case of the calculation of the interaction energy by variation method – SCF procedure ($\Delta E_{AB}(r)$), charge distribution of supermolecule is different from that of isolated molecules A and B, expecially at short distances. This may be one of the reasons for different values of the equilibrium distances calculated by SCF procedure (0.27 nm) and perturbation theory (0.3 nm), on the same level of approximations.

In accordance with the results of [28] it may be assumed that for the system NMAA–benzene the used perturbation scheme overestimates the charge-transfer contribution while the polarization energy is underestimated. It is also known that the attraction contribution from Coulombic forces is comparable in value to the contribution from charge-transfer forces for different types of molecular complexes [29,30]. It may, therefore, be assumed that the real value of the charge-transfer contribution for NMAA–benzene molecular complex is lower than the calculated, but the mutual ratio of the energies of direct and back donation is unaltered.

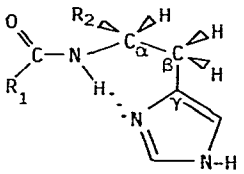
In spite of the problems discussed above, the obtained results show a real possibility of the formation of molecular complexes between peptide bond and the

aromatic nucleus. As for the interaction of NMAA with benzene, results indicating the possibility of the formation of the molecular complex have also been obtained for the interaction of NMAA with imidazole. The interaction with imidazole was studied for two basic geometrical arrangements. In the first case the mid-point of imidazole is situated above the N of peptide bond whereas the nitrogen of the amide group of imidazole is either turned towards or away from the peptide bond (fig. 2a,b). In the second case the mid-point of imidazole is situated above the oxygen of the peptide bond with two different orientations of imidazole (fig. 2c,d).

In locating the imidazole molecule above N of peptide bond with the geometry as described in fig. 2a, the interaction energy is roughly the same as for the system NMAA–benzene in the same geometrical arrangement fig. 1a – see figs. 4a and 6a. However, for the system with the geometrical arrangement fig. 2b, the value of the interaction energy at the same equilibrium distance is roughly the half – fig. 6b.

In locating the imidazole molecule above the oxygen of the peptide bond, the interaction energy of the system in the geometrical arrangement fig. 2d is again roughly twice as the interaction energy of the system in the geometrical arrangement fig. 2c – see fig. 7c,d. In these cases the calculated equilibrium distance of the interacting molecules is, however, very small i.e. 0.21 nm.

It may be assumed that the rotation of the imidazole ring around C_β–C_γ bond in "histidine" peptide bonds is inhibited as a result of intramolecular hydrogen bond formation;



The orientation of the imidazole ring at the formation of molecular complex would then be unambiguously fixed in view of the peptide bond "tosyl hole". In view of the primary specificity of chymotrypsin towards hydrolysis of "phenylalanine" peptide bonds (side chain–benzene) and "histidine" peptide bonds (side chain–imidazole), the formation energy of the complex: peptide bond–imidazole must be less than that of the complex: peptide bond–benzene.

From among the studied geometrical arrangements this requirement is met by the structure of the complex shown in fig. 2a corresponding to the analogous structure of the complex with benzene (fig. 1a).

Despite the fact that the studied systems represent a very rough model without such complex process as specificity of enzymatic process, the theoretical results are comparable to the experiment. The free energy of formation of ES complex between chymotrypsin and methylhydrocynnamate is about –20 kJ/mole [31]. Because methylhydrocynnamate is an ester substrate, the free energy –20 kJ/mole may be correlated with interaction energy calculated for the system benzene–NMAA. In case of amide substrates, the energy of H bond between amide proton of cleaved peptide bond and carbonyl oxygen of Ser 214 enzyme also contributes to the free energy of the ES complex. Calculated values of the interaction energy, however, do not include entropic contributions. Equilibrium distances calculated for the system benzene–NMAA are also in agreement with the experimental value of the distance between the aromatic nucleus of the specific substrates and the peptide bond Trp 215 –Gly 216 of "tosyl hole" chymotrypsin – about 0.3 nm [15].

It therefore appears that the model of the formation of the molecular complex between aromatic nucleus of the side chain of specific substrate and peptide bond "tosyl hole" is capable of suitably describing the primary specificity of chymotrypsin.

The presented results were obtained on a Siemens 4004/150 computer at the computing center of the Comenius University in Bratislava. Calculations were made in double precision accuracy.

References

[1] G.L. Neil, C. Niemann and G.E. Hein, Nature 210 (1966) 903.
[2] K. Martinek, A.V. Levašov and I.V. Berezin, Mol. Biol. (rus.) 4 (1970) 339.
[3] K. Martinek, A.V. Levašov, and I.V. Berezin, Mol. Biol. (rus.) 4 (1970) 517.
[4] I.V. Berezin, A.V. Levašov, and K. Martinek, FEBS Letters 7 (1970) 20.
[5] I.V. Berezin and K. Martinek, Struktura i funkcia fermentov (rus.) (Izd. Mosk. Gosudarstv. Univ., 1972).
[6] J.R. Knowles, J. Theoret. Biol. 9 (1965) 213.
[7] M.B. Hille and D.E. Koshland Jr., J. Am. Chem. Soc. 89 (1967) 5945.

[8] M.L. Bender, G.R. Schonbaum and B. Zerner, J. Am. Chem. Soc. 84 (1962) 2540.
[9] P.B. Sigler, D.M. Blow, B.W. Matthews and R. Henderson, J. Mol. Biol. 55 (1968) 143.
[10] J.J. Birktoft, B.W. Matthews and D.M. Blow, Biochem. Biophys. Res. Comm. 36 (1969) 131.
[11] B.W. Matthews, P.B. Sigler, R. Henderson, D.M. Blow and C.O. Parkes, Nature 214 (1967) 652.
[12] J.J. Birktoft, D.M. Blow, R. Henderson and T.A. Steitz, Phil. Trans. Roy. Soc. London B 257 (1970) 67.
[13] R. Henderson, J. Mol. Biol. 54 (1970) 341.
[14] T.A. Steitz, R. Henderson and D.M. Blow, J. Mol. Biol. 46 (1967) 337.
[15] R. Henderson, Ch.S. Wright, G.P. Hess and D.M. Blow, Cold Spring Harbor Symposia on Quantitative biology, Vol. XXXVI (The Cold Spring Harbor Lab. 1972) p. 63.
[16] J.A. Pople and D.L. Beveridge, Approximate molecular orbital theory (McGraw-Hill 1970).
[17] V. Kvasničkа, V. Lavrinc and I. Hubač, Phys. Rev. A10 (1974) 2016.
[18] V. Lavrinc, Ph.D. Thesis, Slovak Tech. University Bratislava (Czechoslovakia 1972).
[19] S. Martinez-Carrera, Acta Crystalogr. 20 (1966) 783.
[20] D.B. Chesnut and P.E.S. Wormer, Theoret. Chim. Acta 20 (1971) 250.
[21] R.G. Jessaitis and A. Streitwieser, Theoret. Chim. Acta 17 (1970) 165.
[22] R. Arnaud, D.F. Farmond-Baud and M. Gelus, Theoret. Chim. Acta 31 (1973) 335.
[23] A. Dargelos, D. Liotard and M. Chaillet, Tetrahedron 28 (1972) 5595.
[24] B. Nelander, Theoret. Chim. Acta 25 (1972) 382.
[25] J.C. Schug and K.A. Levinson, Theoret. Chim. Acta 37 (1975) 269.
[26] R.E. Bruns, J. Mol. Structur. 36 (1977) 121.
[27] L.A. Carrera and W.B. Person, J. Am. Chem. Soc. 94 (1972) 1485.
[28] P. Bañacký, Chem. Zvesti 33 (1979) 145.
[29] W.A. Lathan, R.G. Pack and K. Morokuma, J. Am. Chem. Soc. 97 (1975) 6624.
[30] H. Umeyama and K. Morokuma, J. Am. Chem. Soc. 98 (1976) 7208.
[31] D.W. Ingles and J.R. Knowles, Biochem. J. 108 (1968) 561.