Quantum chemical study of the relative stability of conformers of antitumour drug — 2-formylpyridine thiosemicarbazone

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Various conformations of 2-formylpyridine thiosemicarbazone (2-FPT) have been studied by the PCILO and PPP semiempirical quantum chemical methods. Derivatives of these compounds exhibit carcinostatic activity. The most stable conformations of the free form of 2-FPT have been found. Using simple models the effect of solvent and effect of complexation of 2-FPT with Fe^{2+} on the conformations of 2-FPT have been evaluated.

Квантовохимическими семиэмпирическими методами PCILO и PPP были изучены возможные конформации 2-формилпиридинтиосемикар-базида (2-FPT). Производные этого соединения имеют канцеростатичес-кие действия. Нашлись самые стабильные конформации для свободной формы 2-FPT. Также было изучено влияние растворителя и образования комплекса 2-FPT с ионами Fe^{2+} на конформации 2-FPT при помощи простых моделей.

In their search for antitumour drugs, *Brockman et al.* [1] discovered that 2-formylpyridine thiosemicarbazone (2-FPT) was active against leukemia in mice. The substances were found to inhibit the effect of the enzyme ribonucleoside diphosphate reductase with Fe^{2+} as cofactor. Since then, many derivatives of 2-FPT [2—4] have been investigated with the aim of finding the most active derivative. For the series of 2-FPT derivatives the activities *in vivo* as well as *in vitro* were determined [4]. Another important question to be solved is the conformation of the free form of 2-FPT as well as the effect of complexation with Fe^{2+} on the conformations. The complexation turned out to play an important role in the mechanism of action [2, 5—7].

In an effort to study the electron structure of the 2-FPT derivative in detail we have concentrated on two goals: i) to investigate the most stable conformations of 2-FPT in the free form and bound to Fe^{2+}; ii) to study the reactivity of the

derivatives of 2-FPT in the free form and in the complex with Fe^{2+} and to find correlation between electron structure and biological activity of these compounds. In this paper we focus our attention on the first problem (*i.e.* the study of the conformations), while the study of the second problem has been published recently [8].

Methods and calculations

Two quantum chemical methods, namely PCILO method and PPP method connected with empirical potentials, were used for the calculations of energy of the individual conformers.

The semiempirical PCILO method was used in the standard CNDO/2 parametrization [9—11].

Since the studied systems are conjugated we used also the PPP method [12, 13] which considers π electrons only and evaluated π energy of the system. The standard parametrization of I_μ and $\gamma_{\mu\mu}$ was used in calculation [14] and standard values of $\beta_{\mu\nu}$ resonance integrals considered between adjacent atoms were used [15].

The total energy calculated by PPP method represents only the energy of atoms which form the π system directly. For this reason it is necessary to consider especially nonbonding interaction of the atoms not belonging to the π system (hydrogen atoms) in the study of conformation of this system. This part of energy can be expressed by means of the empirical pair potentials of Buckingham type. For the pair of nonbonding atoms the interaction energy is evaluated as follows

$$\varepsilon_{pq} = -\frac{C}{r_{pq}^6} + D \exp(-Er_{pq}) \tag{1}$$

Empirical constants C, D, and E are taken from Ref. [16]. The total energy of the system expressed in this way can be written as follows

$$E_T = E + E_{rep}^{core} + E_{nb} = \frac{1}{2}\sum_\mu\sum_\nu P_{\mu\nu}(h_{\mu\nu} + F_{\mu\nu}) +$$

$$+ \sum\sum_{\mu<\nu} Q_\mu Q_\nu \gamma_{\mu\nu} + \sum_p\sum_q \varepsilon_{pq} \tag{2}$$

where the first two terms correspond to the energy from PPP method, the third term represents the energy of the nonbonding interactions.

We were interested in the study of formation of complex of 2-FPT with bivalent ions (*e.g.* Fe^{2+}) and its effect on the stability of incidental conformers. Due to computer and computational problems (divergence of the SCF procedure) in the calculation of the complex by the PCILO method we were forced to use approximative model. The complex was studied by PPP method only. Fe^{2+} ion was
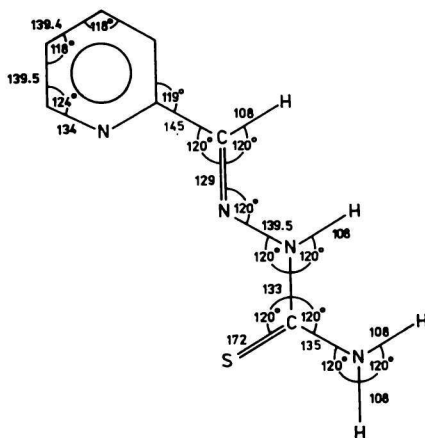
simulated by point charge which represents only electrostatic contribution. Moreover, we have considered nonbonding interaction between Fe^{2+} ion and atoms of 2-FPT. Since the constants C, D, E are not known directly for Fe^{2+} we have used nonbonding potential of argon [17] which gives a true picture of nonbonding of Fe^{2+}.

Within the framework of PPP method we have estimated also the effect of solvent on the individual conformers of the free 2-FPT. The solvation energy can be approximately represented by coulombic part which can be expressed as follows [18]

$$E_{solv}^{coul} = -\frac{1}{2}\left(1 - \frac{1}{\varepsilon}\right) \sum_{\mu} \sum_{v} Q_{\mu}Q_{v}\gamma_{\mu v} \qquad (3)$$

where ε is a relative permittivity of solvent; Q_{μ}, Q_{v} are charges on the atoms μ and v; $\gamma_{\mu v}$ is a bielectron repulsion integral.

In the study of the individual conformers the experimental bond lengths and bond angles were used [19] (Fig. 1).
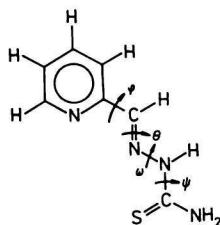

Fig. 1. Geometry of the free form of 2-FPT used in calculations. Bond lengths are given in pm.

A. Study of conformers of the free form of 2-FPT by PCILO method

In thiosemicarbazone chain we have aimed at the study of the rotations around four bonds (Fig. 2). Variation of these four dihedral angles leads to sixteen energetic minima corresponding to the stable conformers. In ten conformations some atoms are approached so close that their nonbonding repulsion causes that the total energy is much higher than in additional six conformations. Such unstable

Fig. 2. Dihedral angles varied in calculations.

conformers are obtained especially by the rotation around N—N bond (for $\omega \to 180°$) where the strong repulsion between S and H atoms bonded on C-7 occurs.
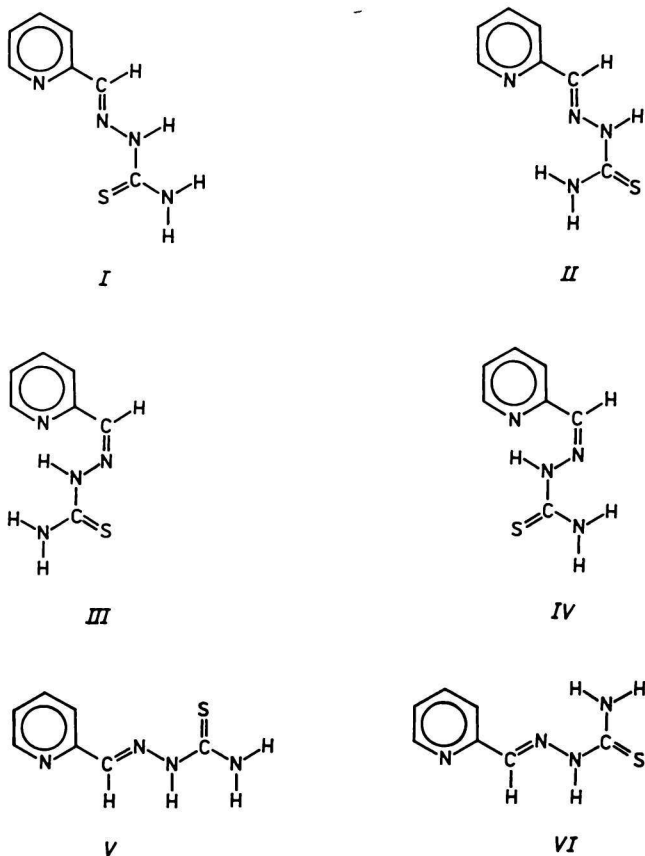

Fig. 3. Stable planar conformations of the free form of 2-FPT.

For this reason we present here only six more stable conformers (Fig. 3). All these conformers are planar. Energies of these conformers and their relative occurrence calculated from Boltzmann distribution at $T = 310$ K are given in Table 1.

Table 1

Total energy of individual conformers of the free form of 2-FPT calculated by PCILO method and their relative occurrence

Conformation[a]	E_T/kJ mol^{-1}	Relative occurrence[b] in %
I	− 298 049.4	0.00
II	− 298 075.0	99.85
III	− 298 034.4	0.00
IV	− 298 056.3	0.07
V	− 298 039.7	0.00
VI	− 298 056.4	0.07

a) For the designation of conformers see Fig. 3.
b) Calculated from Boltzmann distribution at 310 K.

Transitions between conformations always have energetic barrier. Maximum energy lies mostly at twist angle 90°. Such rotations have been studied by the change of twist angle with the step of 30°. The total twisting of double bond (C=N) has not been considered in calculations because at twist angle $\Theta = 90°$ biradical state arises which cannot be described correctly by the used method. That is why we have calculated for this case only the energy of *cis* and *trans* conformers and the conformers with near value of Θ.

For rotation of φ (*i.e.* transition from conformation *II* to *VI*) we have found energetic barrier 25.7 kJ mol^{-1}, for rotation of ψ (from *II* to *I*) 142.4 kJ mol^{-1} and for rotation of ω the energetic barrier is 151.3 kJ mol^{-1}. During the last mentioned rotation the second minimum of energy is near to $\omega = 150°$, however, this minimum is by 85 kJ mol^{-1} higher than that corresponding to the structure *I*.

B. Study of conformers of the free form of 2-FPT by PPP method

The same calculations, as we had described above, have been carried out by PPP method. The energy of nonbonding interaction of the atoms not belonging to the π system has been added to the energy of PPP method. Results of calculations for the individual conformations and their relative occurrence are given in Table 2.

Total energy of individual conformers of the free form of 2-FPT calculated by PPP method connected with empirical potentials

Conformation[a]	$E_T/kJ\ mol^{-1}$	Relative occurrence[b] in %
I	− 19 868.5	0.86
II	− 19 879.4	60.25
III	− 19 861.5	0.01
IV	− 19 861.5	0.06
V	− 19 871.1	2.41
VI	− 19 878.1	36.39

a, b) See Table 1.

Table 3

Values of solvation and summary energies calculated by PPP method connected with empirical potentials for individual conformers and their relative occurrence expressed with regard to E_{SUM}

Conformation[a]	$E_s/kJ\ mol^{-1}$	$E_{SUM}{}^c/kJ\ mol^{-1}$	Relative occurrence[b] in %
I	− 197.6	− 20 066.1	0.05
II	− 206.1	− 20 085.5	80.90
III	− 204.7	− 20 061.8	0.01
IV	− 208.0	− 20 069.5	0.16
V	− 200.2	− 20 071.3	0.34
VI	− 203.6	− 20 081.7	18.54

a, b) See Table 1.
c) Summary energy is expressed as $E_{SUM} = E_s + E_T$.

The above-mentioned calculations correspond to isolated molecules *in vacuo*. With regard to the fact that the derivatives of 2-FPT exist in polar liquid phase, we estimated the solvation energy E_s by the PPP method according to eqn *(3)*. Summary energy E_{SUM} and corresponding relative occurrence (Table 3) was obtained by adding the solvation energy E_s to the energy of isolated molecule E_T.

C. Conformation study of 2-FPT … Fe^{2+} complex

In the calculation of complex 2-FPT … Fe^{2+} the experimental geometry was used [19] (Fig. 4). This geometry was the basis also for the calculations of the other
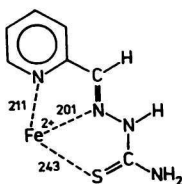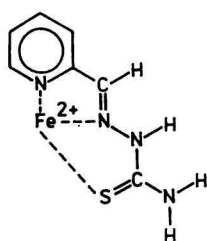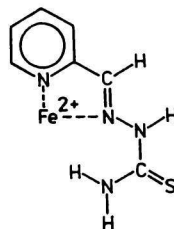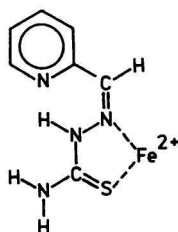
Fig. 4. Geometry of complex 2-FPT with Fe^{2+} used in calculation. Bond lengths are given in pm.
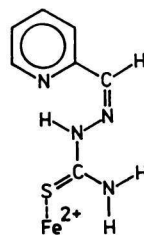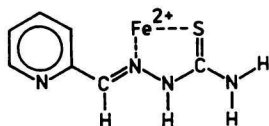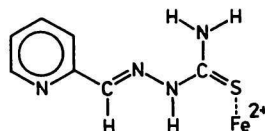

Fig. 5. Stable planar conformations of complex 2-FPT ... Fe^{2+}.

conformations, at which the position of Fe^{2+} was optimalized and the whole system was considered to be plane. The individual complexes of conformers are shown in Fig. 5. Energy of complexes has been calculated by PPP method where Fe^{2+} was

Table 4

Total energy of individual conformers of complex 2-FPT ... Fe^{2+} calculated by PPP method connected with empirical potentials

Conformation[a]	$E_T/\text{kJ mol}^{-1}$	Relative occurrence[b] in %
I	− 20 219.5	99.99
II	− 19 762.1	0.01
III	− 20 041.6	0.01
IV	− 20 025.4	0.01
V	− 19 958.5	0.01
VI	− 20 033.8	0.01

a, b) See Table 1.

represented by a point charge. The energy of nonbonding interactions including the interaction of Fe^{2+} with atoms of 2-FPT has been added to the π energy. Such total energy and the relative occurrence of individual complexes is given in Table 4.

Discussion

In this study we try to evaluate relative stability of the free form of derivatives of 2-FPT. The effect of solvent and effect of complexation on the conformation stability have been estimated. Two semiempirical methods were used. The first PCILO method is one of the most appropriate methods for the study of conformations. The second was PPP method with nonbonding potentials. In spite of crude simplification considered in this method we found that the results obtained by this method are qualitatively similar to those reached by PCILO method. Such agreement is not surprising if we take into account the fact that 2-FPT system is conjugated and the most stable conformations are planar. The main differences between energies of individual conformers are in π-electron energy and energy of nonbonding interactions. The both methods indicate the conformation *II* to be most stable and conformation *VI* as the second most stable. Evaluation of the relative occurrence by Boltzmann distribution at 310 K shows that the free form of 2-FPT is prevailingly in the conformation *II* (60—98 %) depending on the used method. The rest is in conformation *VI* while the occurrence of other conformers is negligible. Consideration of solvent effect in the calculations shows that the polar medium still more increases the relative occurrence of conformation *II*.

Our results contradict the assumption of *French* and *Blanz* [20] who postulated existence of 2-FPT in conformation *IV*. On the other hand, our results agree well with recent findings [7] where measuring of ^{1}H-n.m.r., u.v., and i.r. spectra of the free form of 2-FPT at ambient temperature shows that the free form of 2-FPT

prevalently exists in conformation *II*, partly in conformation *VI*. The occurrence of conformation *IV* is very small. It has turned out that the transition from conformation *II* to *VI* (angle φ) has very low energetic barrier. Transition from conformer *II* to *I* (rotation ψ) has already relatively high energetic barrier.

In the study of the effect of complexation on the conformation of 2-FPT we were not able to use PCILO method. That is why we used the PPP method where Fe^{2+} was simulated by a point charge and also nonbonding interaction was included in the calculation. Such approach is very crude because it describes only coulombic and nonbonding part of the total interactions. In spite of this fact at least the qualitative conclusions can be drawn. After the forming of complex all conformations change to conformation *I* which has higher occurrence than 99 %. It seems logical because only in this conformation the Fe^{2+} ion bonds directly on the lone pairs of the free atoms (N, N, S). These results agree also with experimental measurements of complexes 2-FPT ... Fe^{2+} in solution [7] and also with X-ray analysis in solid crystals [19].

The conclusions of present study can be used in the further theoretical and experimental study of the properties of these compounds and also in the study of mechanism of their carcinostatic effects [8].

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Translated by S. Miertuš