ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN – POLONIA
VOL. XXIII, N 4, 3 SECTIO DDD 2010

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Theoretical studies on keto-enol tautomerism, gas phase acidity and spectral properties of phenylbutazone

Badania obliczeniowe tautomerii keto-enolowej, kwasowości w fazie gazowej oraz właściwości spektralnych fenylobutazonu

INTRODUCTION

Phenylbutazone (4-butyl-1,2-diphenylpyrazolidine-3,5-dione) is an anti-inflammatory and analgesic drug invented in 1948 and introduced into human medicine in 1949. Despite its several important adverse effects, it is still used, mainly externally, in many countries. Two comprehensive reviews on its action and adverse effects are given in the literature [3, 4].

From the chemical point of view, an interesting property of this drug is a special kind of keto-enol tautomerism. The proton located in the fourth carbon of pyrazolidine ring has an acidic property and can form an enol with the neighbouring keto group forming 4-butyl-5-hydroxy-1,2-diphenyl-1,2-dihydro-3H-pyrazol-3-one. This implies that phenylbutazone can act as a very weak acid and thus can be directly titrated with sodium hydroxide in semi-acidic medium. This method of quantitative determination is official in the 6th edition of the Polish Pharmacopoeia [8] and is extensively discussed [2].

To the best of our knowledge, the molecular modelling of this drug is missing in the literature. Additionally, there is not any paper related explicitly to the keto-enol tautomerism in any other sense. This fact inspired us to perform a computational study of phenylbutazone molecule, investigating keto-enol tautomerism, both in gas and solvent phases, followed by spectral properties modelling. The simulated UV and IR spectra are compared with the experimental ones.

EXPERIMENTAL DESIGN

All computations were done inside Gaussian 09 [7] working under GNU/Linux on a computational cluster. Initial conformational analysis was done inside MOE 2007.09 (Chemcomp, USA). Visualization of the results was performed with Gabedit package [6].

Phenylbutazone was purchased from Sigma-Aldrich (St. Louis, USA). The IR spectrum was recorded in attenuated total reflectance (ATR) mode using Thermo Scientific Nicolet 6700IR spectrophotometer. For the UV spectra recording, a 10 mg/L solution in methanol (spectroscopic grade, POCH, Gliwice, Poland), water (deionised with Milipore system) and 0.1 mol/l NaOH was prepared. Then, the spectra were recorded using a Hitachi UV-2001 double-beam spectrophotometer against the respective blank solvent.

RESULTS AND DISCUSSION

Initial geometries of keto, enol and anion forms of phenylbutazone were found with the conformational studies done inside MOE (using all possible techniques). The geometries with the lowest energy were chosen for further optimization inside Gaussian at B3LYP/6-31g(d) level in the gas phase. The optimized geometries of these three forms are depicted in Figures 1–4 with distances between atoms given in angstroms.

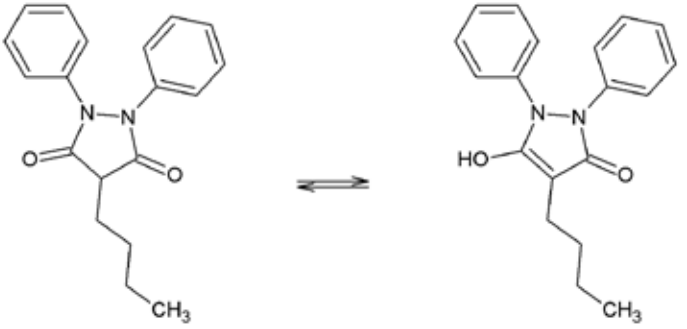

Fig. 1. The keto-enol tautomerism of the phenylbutazone

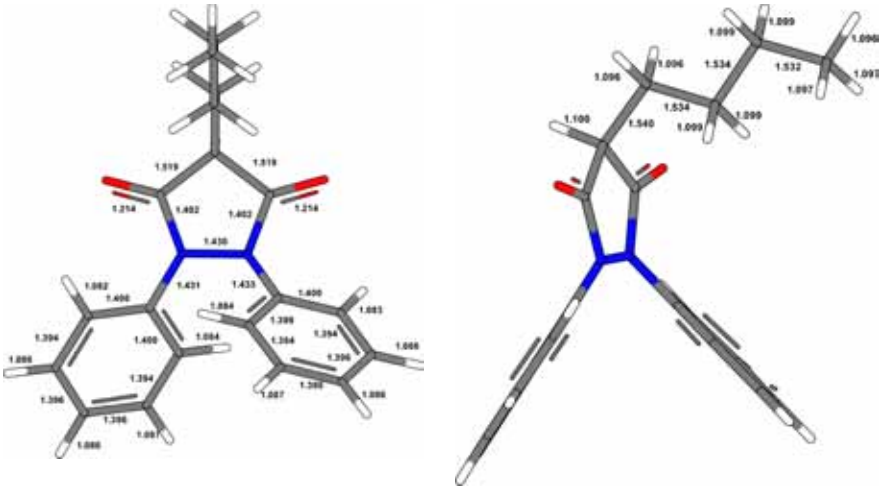

Fig. 2. The structure of keto form of phenylbutazone optimized at B3LYP/6-31g(d) level with distances given in angstroms

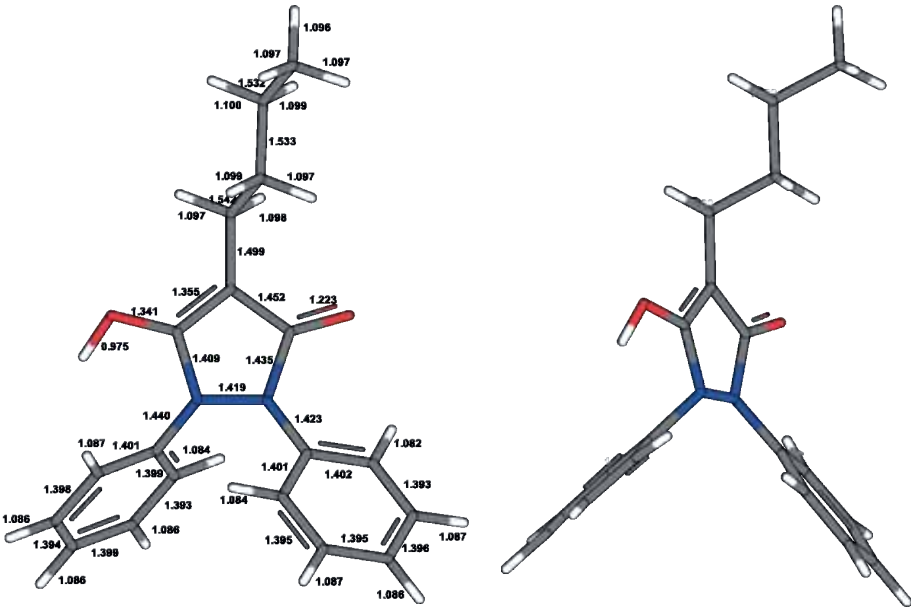

Fig. 3. The structure of enol form of phenylbutazone optimized at B3LYP/6-31g(d) level with distances given in angstroms

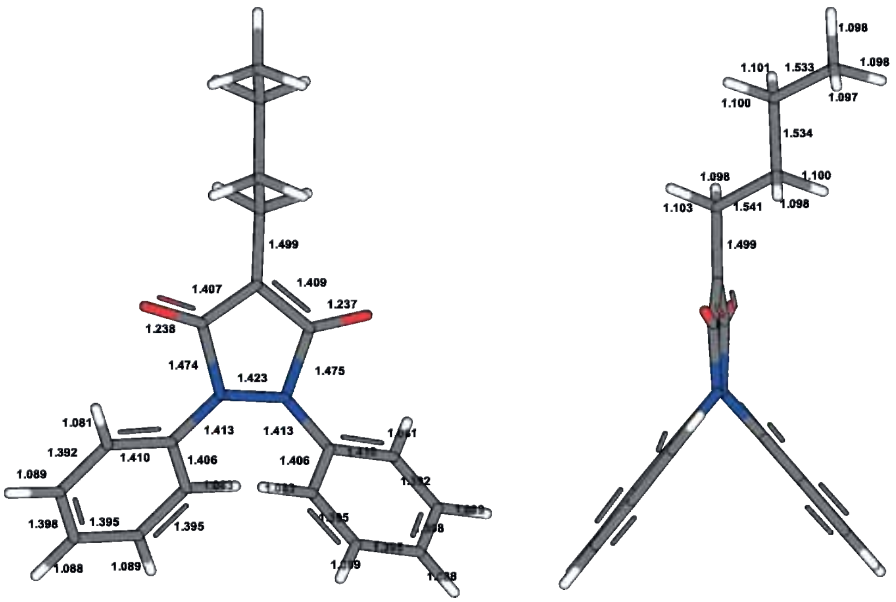

Fig. 4. The structure of anionic form of phenylbutazone optimized at B3LYP/6-31g(d) level with distances given in angstroms

The optimal geometry of keto form (Fig. 2) has the butyl chain located in perpendicular position to the pyrazolidine ring, almost symmetrically against a ring plane symmetry. Two phenyl rings are also located symmetrically, slightly skewed. The distances between atoms in pyrazolidine ring are symmetrical. The C-(C=O)-N angles are equal to 107.5° and 107.6°, the (C=O)-C-(C=O) angle is equal to 104.12°. Nitrogen angles are equal to 109.9° and 110.3°. The pyrazolidine ring is almost flat and the highest dihedral angle is equal to 5.7°. The phenyl rings are reflexed outside a pyrazolidine plane in angles 30.5° and 30.1°. The buthyl chain is connected with a plane an angle 49.2°.

The optimal geometry of enol form (Fig. 3) differs mainly with buthyl chain location and assymetry of dihydropyrazol ring. The bonds at enol side of dihydropyrazol ring are shorter, whereas the enol bond between carbon and oxygen is longer. The C-(C-OH)-N angle is a bit wider – equal to 112.6°, whereas the C-(C=O)-N one is equal to 106.1° only. The dihydropyrazol ring is also less flat, with the highest dihedral angle equal to 8.2°. The phenyl substituents angles are increased at enol side (41.6°) and slightly decreased in keto side (27.2°). The butyl chain, due to impresence of the hydrogen in 4- position of dihydropyrazol ring, lies almost in a ring plane (2.9° angle).

The dissociation process (anion form is depicted in Fig. 4) strenghtens all bonds of dihydropyrazol ring and makes it more flat (the widest dihedral angle is equal to 3.3°). The angle between the plane and the phenyl ring at enol side increases to 40.0° due to the absence of hydrogen atom.

The optimized keto and enol geometries were used in transitional state prediction performed by QST2 method (Fig. 5). The optimized transitional state demonstrated one imaginary frequency equal to -2101 cm^{-1} of very high intensity (1069.89) associated with a proton transfer path. The distance between proton and carbon is equal to 1.49 Å, between proton and oxygen – 2.31 Å. These atoms form an angle 108.2°. The proton is located at 40.4° angle outside the ring plane whereas the enol oxygen at 24.7° angle. The transitional angle of buthyl chain (to the plane) is equal to 39.1°. The presence of the proton outside the plane causes a change in phenyl substituent position by about 10° to the ring plane in opposite direction to the proton position.

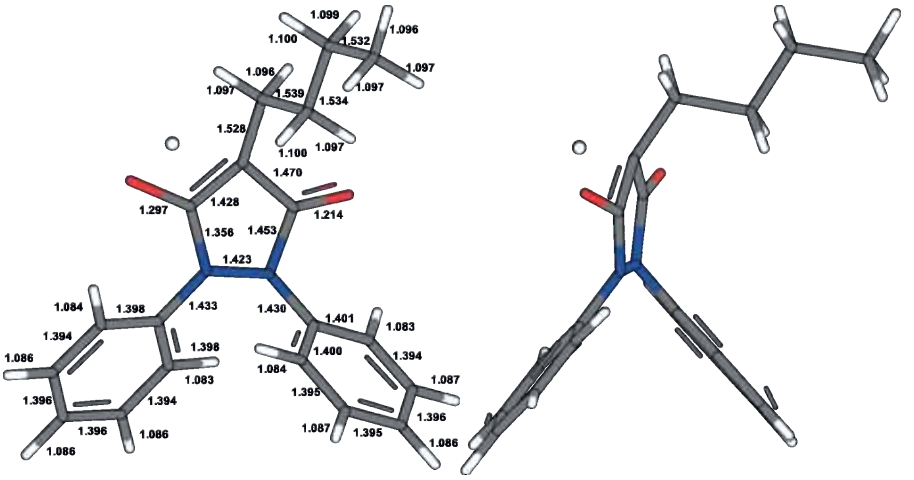

Fig. 5. The structure of transition state between keto and enol forms of phenylbutazone, optimized by QST2 method at B3LYP/6-31g(d) level with distances given in angstroms

The vibrational analysis done at B3LYP/6-31g(d) level allows to compute differences in energy, enthalpy and Gibbs free energy. The energetic differences between keto form and other geometries were then computed at higher basis set: B3LYP/6-311++g(d,p) in a gas phase and with SCRF model in methanol and water. To investigate the effect of the theoretical level, both basis sets were also used with BP86 and MP2 methods in a gas phase. The MP2 method was also computed with cc-pVDZ and cc-pVTZ basis sets.

Table 1. Energies (kJ/mol) relative to keto form computed at different levels

	Enol	TS	Anion
B3LYP/6-31g(d)	55.31	299.9	1421.72
Zero-point correction	-0.99	-15.78	-35.54
Thermal correction to Energy	-0.32	-16.3	-36.34
Thermal correction to Enthalpy	-0.32	-16.3	-36.34
Thermal correction to Gibbs Free Energy	-1.63	-13.84	-32.13
B3LYP/6-311++g(d,p)	40.97	291.41	1382.86
B3LYP/6-311++g(d,p) (Methanol SCRF)	35.57	293.98	-
B3LYP/6-311++g(d,p) (Water SCRF)	36.53	294.11	-
BP86/6-31g(d)	52.16	267.01	1403.35
BP86/6-311++g(d,p)	38.39	259.07	1367.02
MP2/6-31g(d)	68.29	384.45	1455.48
MP2//6-311++g(d,p)	52.09	369.71	1430.65
MP2/cc-pVDZ	52.55	365.28	1443.63
MP2/cc-pVTZ	47.42	363.97	1439.07

The results (Table 1) are quite similar – enol form has energy about 50 kJ/mol higher than keto form and its transitional state is about 300 kJ/mol higher than keto form. Incorporating more complex basis set 6-311++g(d,p) makes the difference between keto and enol forms slightly lower whereas it almost does not change the computed energetic difference between keto and transitional states. Incorporating the solvent presence into the computation lowers the energy difference between keto and enol forms even more to value around 35 kJ/mol, similarly to both methanol and water. The BP86 method gives a lower difference than B3LYP whereas MP2 method results in visibly higher values.

The obtained energetic differences are comparable to those presented by Delchev for barbituric acid [5] at B3LYP/D95** level: 82, 63 and 45 kJ/mol (depending on keto group involved). However, the energetic levels of transitional states for barbituric acid are visibly lower: 202, 194, 268 kJ/mol, respectively.

The energetic difference between anion and keto forms can be treated as the energy of the deprotonation of phenylbutazone (as energy of the proton is equal to zero). The lowest value 1367.02 kJ/mol is obtained with BP86/6-311++g(d,p) whereas the highest 1455.48 kJ/mol is obtained with MP2/6-31g(d). The difference in enthalpy at B3LYP/6-31g(d) level is equal to 1330.39 kJ/mol.

Taking into account the proton enthalpy equal to 5/2RT (6.20 kJ/mol at 298 K) [1], the enthalpy of deprotonation (proton affinity of the anion) is equal to 1336.59 kJ/mol. The difference in Gibbs free energy is equal to 1335.91 kJ/mol. Introducing the proton free energy (equal to -26.7 kJ/mol), free energy of deprotonation (gas phase acidity) is equal to 1362.89 kJ/mol.

The simulated IR spectra at B3LYP/6-311g(d) level of keto and enol forms are presented with the experimental one in Fig. 6. It can be generally concluded that the experimental spectrum is much closer to keto form. The enol form spectrum contains two strong bands not present in the experimental spectrum. The one is located at 3660 cm^{-1} and is caused by enol O-H stretching, the second is located at 1439 and 1442 cm^{-1}. The last two resonances are caused by the outside and inside movements (in opposite sides) of two carbon atoms forming a double bond in a dihydropyrazol ring. This movement is in resonance with butyl chain, causing specific hydrogen movements inside. On the contrary, there are no significant bonds in keto form (and in experimental spectrum) between 1400 and 1500 cm^{-1}.

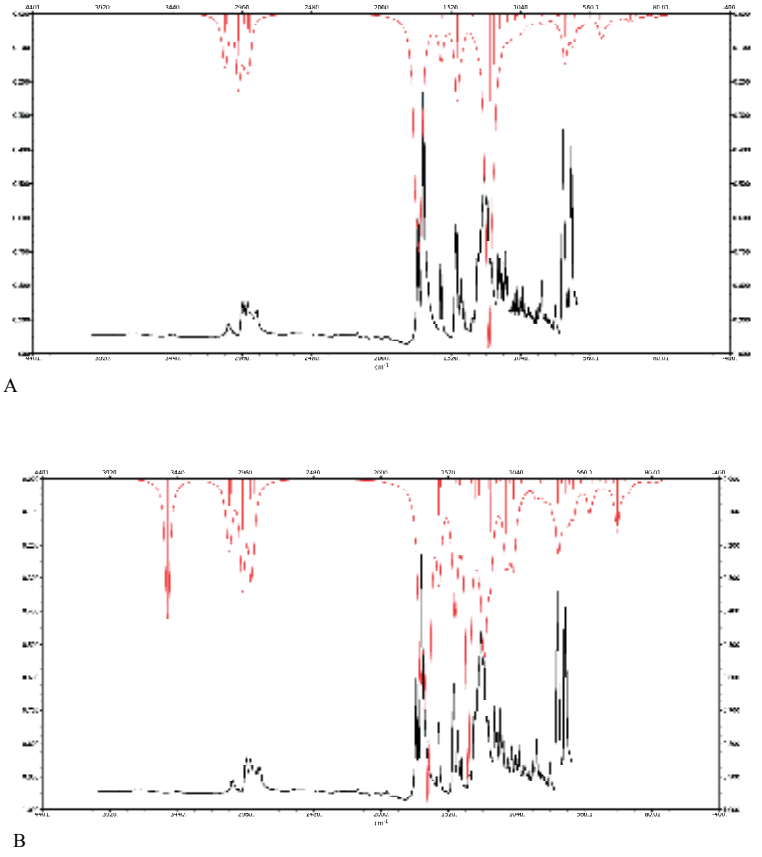

Fig. 6. The simulated spectra of keto (A) and enol (B) forms of phenylbutazone, computed at B3LYP/6-31g(d) level and scaled by factor 0.96. As an overlay, experimental ATR-FTIR spectrum is presented (at the bottom)

The most important (strongest) IR absorption bands in the experimental spectrum were interpreted by analyzing the vibrational vectors at B3LYP/6-31g(d) level. These are:

1. Several resonances above 2800 cm^{-1} caused by C-H stretching. These resonances form two visible groups. One, above 3000 cm^{-1} on the experimental spectrum, is caused by phenyl rings, the second and more intense, below this wavenumber, is caused by the butyl chain.
2. 1750 cm^{-1}, caused by stretching of C=O bonds in the same directions.
3. 1713 cm^{-1}, caused by stretching of C=O bonds in the opposite directions.
4. 1595 cm^{-1}, caused by carbon atom movements inside phenyl rings, following the edge, in opposite directions.
5. 1486 cm^{-1}, caused by hydrogen atom movements inside phenyl rings, following the edge, in the same directions.
6. 1293 cm^{-1}, caused by several resonances of pyrazolidine ring (not affecting planarity), involving the butyl chain.
7. 753 cm^{-1}, caused by bending of the hydrogens outside a phenyl ring plane and in-plane movement of the pirazolidine carbon atom with attached bytyl chain.
8. 694 cm^{-1}, caused by similar bending of the hydrogens outside a phenyl ring plane, without the carbon movement.

To simulate the ultraviolet spectra, a time dependent density functional theory computation (TD-DFT) was carried out at B3LYP/6-311++g(d,p) in a gas phase, methanol and water, solving for 25 excited states. The visualization of HOMO electron orbitals of three forms is presented in Fig. 7 whereas LUMO orbitals are depicted in Fig. 8.

The HOMO and LUMO orbitals in keto form are located symmetrically along the molecule. The excitation occurs at 4.2896 eV (289 nm) in the gas phase. The enol form has the orbitals assymetrical and HOMO is located mainly at the side with C=O group, whereas LUMO at the side with C-O-H group. The energy of HOMO-LUMO excitation in the gas phase lowers to 4.0282 eV (304 nm). Anionic form has HOMO located mainly in a dihydropyrazol ring and LUMO is located far away of the molecule. The energy of the excitation of this transition is significantly lower: 3.3631 eV (368 nm).

The simulated UV spectra are shown in Figure 9. It can be concluded that the solvent has a very weak influence onto the spectrum of keto form and these spectra almost do not differ. Moreover, all the spectra simulated in methanol are very similar to these simulated in water. In the case of enol form, solvent introduction increases HOMO-LUMO excitation energy, shifting 304 nm band to ~270 nm which results in disappearance of any significant absorption above 300 nm. The anion form has the strongest difference between gas phase and solvent spectra. The HOMO-LUMO band at 368 nm and several minor bands are shifted in a similar manner below 300 nm in the presence of both, methanol and water. The gas phase spectrum has no peaks below 260 nm whereas solvent shifts have many of the peaks into this region.

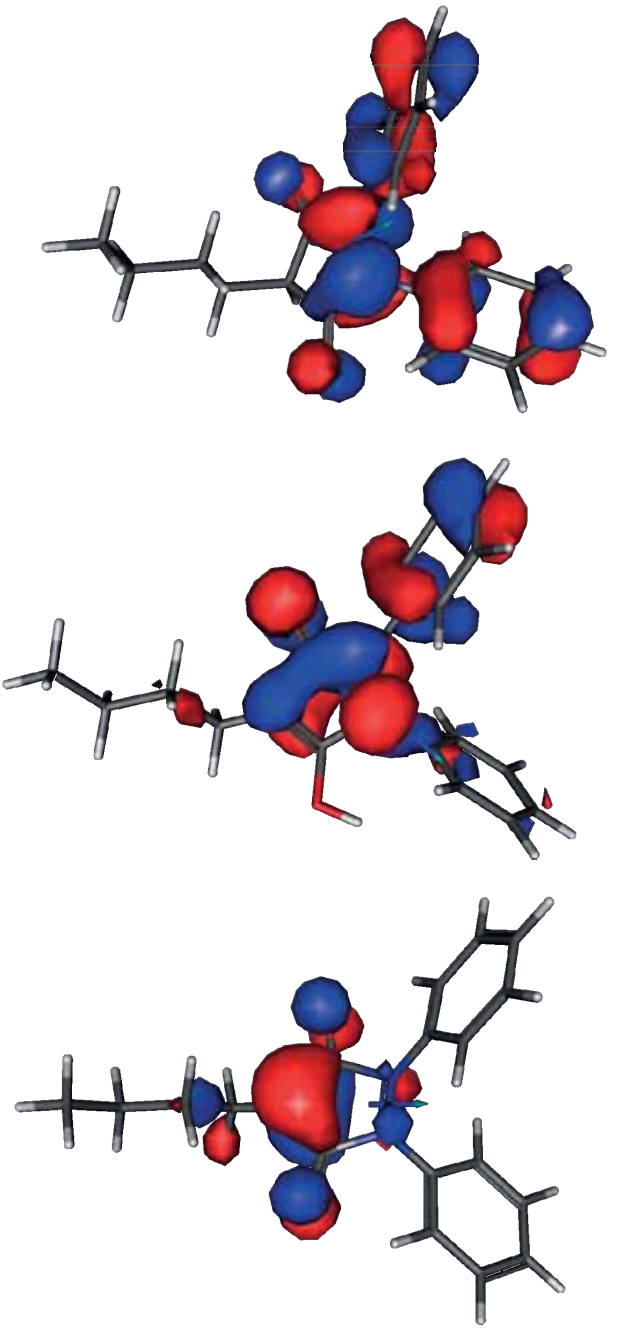

Fig. 7. The highest occupied molecular orbitals (HOMO) of keto(A), enol(B) and anion(C) forms of phenylbutazone

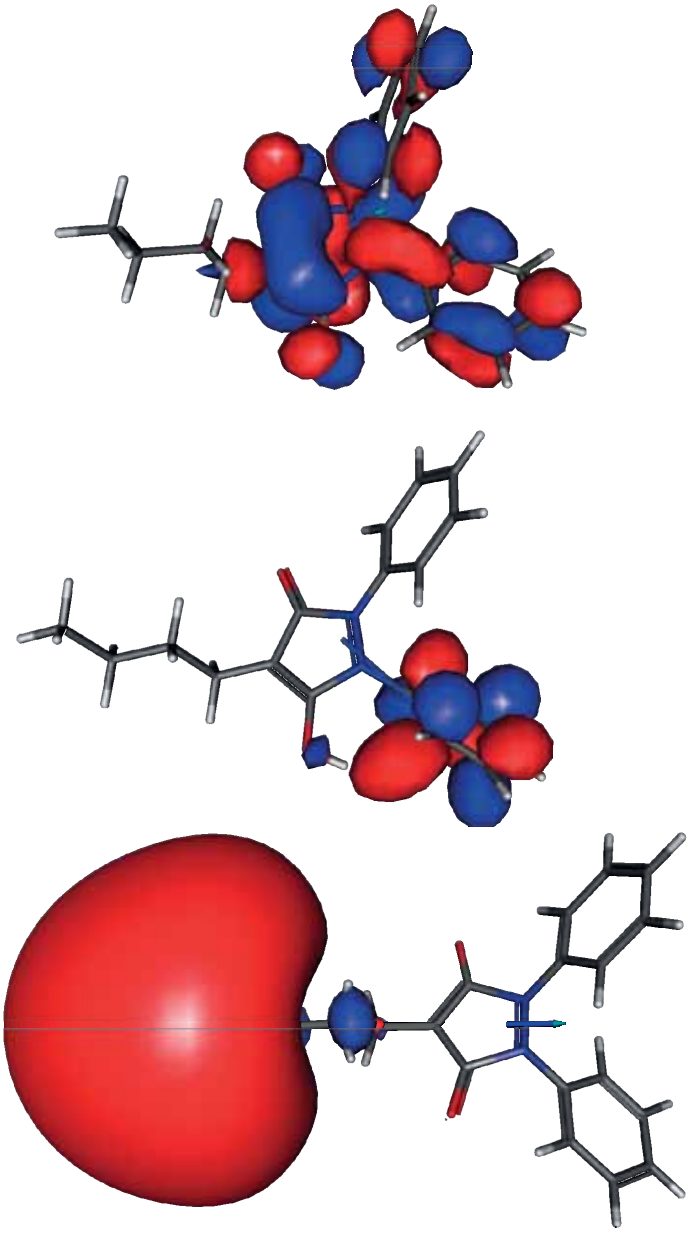

Fig. 8. The lowest unoccupied molecular orbitals (LUMO) of keto(A), enol(B) and anion(C) forms of phenylbutazone

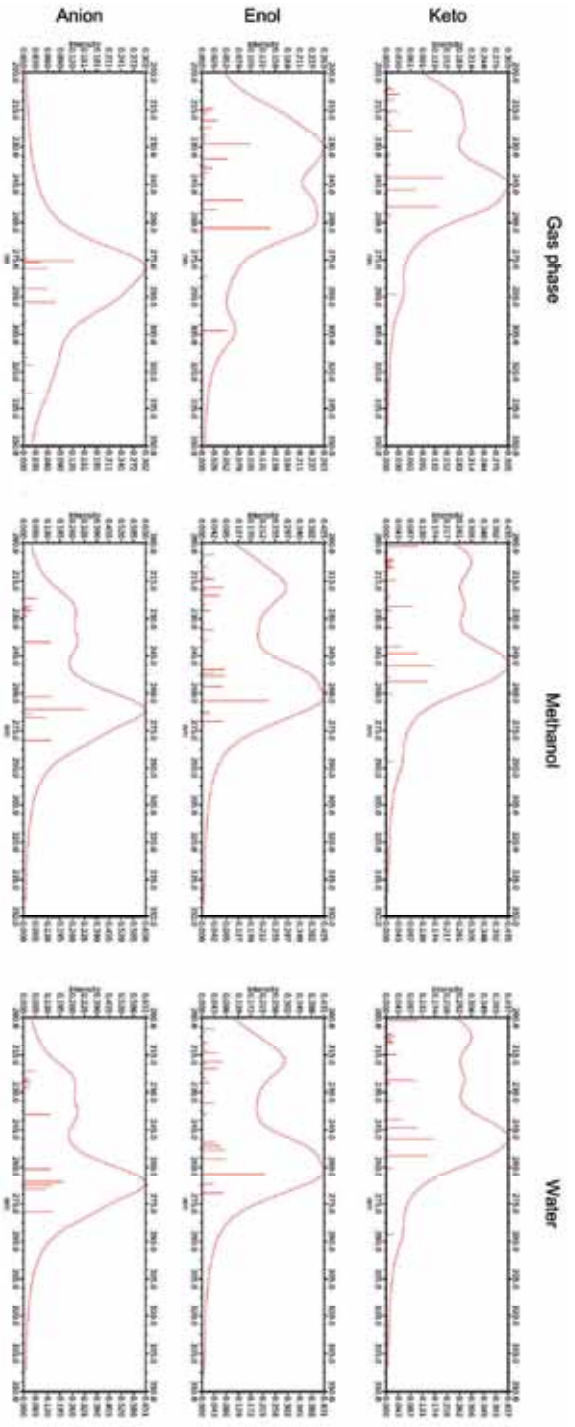

Fig. 9. Simulated UV spectra of three forms of phenylbutazone in gas phase, methanol and water

Comparing the theoretical spectra with the experimental ones, it can be concluded that the experimental spectrum of phenylbutazone dissolved in methanol is similar to the mixture of all three theoretical spectra (Fig. 10). Therefore, it cannot be clearly stated which form is dominant in this solvent and there is a possibility that all of them exist in an equilibrium state. However, there is good agreement (Fig. 11) of the theoretical anion spectrum with the experimental spectrum in NaOH solution which confirms dissociation of phenylbutazone and the existence in this solution mainly in the anionic form.

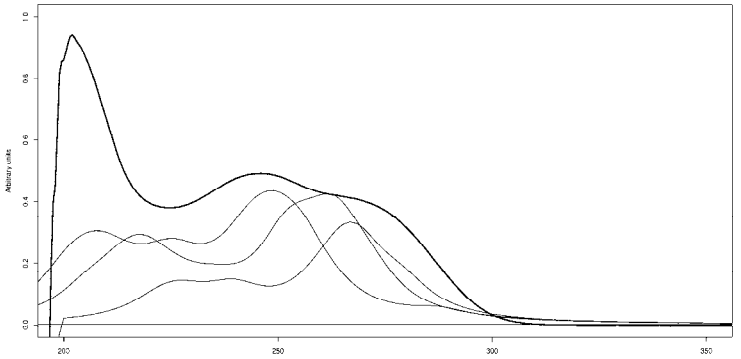

Fig. 10. The simulated spectra (SCRF, methanol) of keto, enol and anion forms of phenylbutazone (thin lines) with an experimental spectrum of phenylbutazone (in methanol)

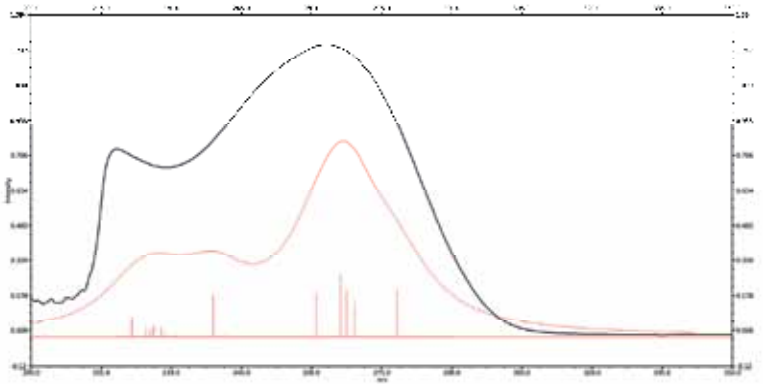

Fig. 11. The simulated spectrum of phenylbutazone anion with an experimental spectrum of phenylbutazone in NaOH solution

CONCLUSIONS

The obtained energetic differences between keto and enol forms of phenylbutazone are comparable to other compounds exhibiting keto-enol tautomerism. However, the transition barrier is visibly higher, comparing – for example – with barbituric acid. The simulated UV and IR spectra have significant differences between keto, enol and anion, allowing estimation which form is present in crystal and the solution. The spectra very closely agree with the experimental ones, confirming the existence of keto form mainly in the crystal substance and anion form in NaOH solution. Methanolic solution of phenylbutazone contains probably three forms in some equilibrium.

Acknowledgement. The authors gratefully acknowledge the computational support (access to the cluster) of Interdisciplinary Centre for Mathematical and Computational Modelling, Warsaw University, Poland, under the computational grant No. G36-8.

REFERENCES

1. Bartmess J. E.: Thermodynamics of the Electron and the Proton. J. Phys. Chem., 98, 6420, 1994.
2. Beckett A.H., Stenlake J. B.: Practical Pharmaceutical Chemistry, Vol. 1, The Athlone Press, London, 1975, pp. 137
3. Brooks P.M., Day R.O.: Nonsteroidal antiinflammatory drugs - Differences and similarities. New Engl. J. Med., 324, 1716, 1991.
4. Clive D.M., Stoff J.S.: Renal syndromes associated with nonsteroidal antiinflammatory drugs. New Engl. J. Med., 310, 563, 1984.
5. Delchev, V.B.: DFT ab initio study of the keto-enol tautomerism of barbituric acid. J. Struct. Chem. 45, 570, 2004.
6. Frisch M.J.; Trucks, G.W., Schlegel, H.B. et al., Inc., Wallingford CT, 2009.
7. http://gabedit.sf.net/
8. Polish Pharmacopoeia, 6th Edition, pp. 572.

SUMMARY

The keto, enol and anion forms of phenylbutazone were optimized in a gas phase at B3LYP/6-31g(d) level with vibrational analysis. The transitional state between keto and enol forms was estimated and optimized in the same way. The energetic differences between these forms were evaluated in a gas phase and with SCRF in methanol and water with B3LYP, BP86, MP2 and HF methods, using 6-31g(d) and 6-311++g(d,p) basis sets. MP2 calculations were also performed with cc-pVDZ and cc-pVTZ basis sets for comparison. The keto form has the lowest energy and the difference between keto and enol is about 50 kJ/mol. The transitional barrier is about 300 kJ/mol above keto form. Anionic form is about 1400 kJ/mol higher than keto form. The presence of the solvent lowers the energetic difference. The gas phase acidity (B3LYP/6-31g(d)) is equal to 1362.89 kJ/mol. The vibrational spectra were interpreted and compared. The ATR-FTIR experimental spectrum is very close to the theoretical one of keto form, indicating that this form is mainly present

in the crystals. Next, the TD-DFT study was carried out, visualizing orbitals and predicting UV spectra in a gas phase, methanol and water. The experimental UV spectrum in methanol has a shape similar to mixed theoretical spectra of keto, enol and anion. The experimental UV spectrum in NaOH solution is very close to the anion theoretical spectrum, confirming dissociation in this solvent.

Keywords: phenylbutazone, keto-etnol tautometism, ultraviolet spectrum, infrared spectrum

STRESZCZENIE

Tautomeryczne formy fenylobutazonu: ketonowa i enolowa oraz anion fenylobutazonu zostały poddane optymalizacji na poziomie B3LYP z użyciem bazy 6-31g(d) z dodatkową analizą wibracyjną. Otrzymano również geometrię stanu przejściowego pomiędzy formami. Różnice w energii tych form zostały obliczone w fazie gazowej oraz z zastosowaniem metody SRCF w fazie ciekłej – metanolu i wodzie. Różnice energetyczne wyliczono dla tych geometrii na poziomach B3LYP, BP86, MP2 i HF z użyciem baz 6-31g(d) i 6-311++g(d,p), a dla MP2 dodatkowo z bazami cc-pVDZ i cc-pVTZ. Forma ketonowa ma energię niższą od enolowej o ok. 50 kJ/mol. Stan przejściowy jest zlokalizowany ok. 300 kJ/mol wyżej od formy ketonowej. Forma anionowa ma energię ok. 1400 kJ/mol wyższą od ketonu. Wprowadzenie rozpuszczalnika wyraźnie obniża te różnice. Kwasowość w stanie gazowym na poziomie B3LYP/6-31g(d) wynosi 1362.89 kJ/mol. Analiza wibracyjna pozwoliła na otrzymanie teoretycznych widm IR, które zostały zinterpretowane. Widmo eksperymentalne ATR jest zgodne z formą ketonową, co świadczy o istnieniu prawie wyłącznie tej formy w krystalicznej substancji. Dodatkowo przeprowadzono analizę TDDFT, wizualizując orbitale elektronowe i otrzymując teoretyczne widma UV w stanie gazowym, metanolu i wodzie. Widmo eksperymentalne w metanolu ma kształt mieszaniny widm teoretycznych ketonu, enolu i anionu. Natomiast widmo eksperymentalne w roztworze NaOH jest zgodne z widmem anionu w metanolu, co świadczy o praktycznie całkowitej dysocjacji związku w tym rozpuszczalniku.

Słowa kluczowe: fenylobutazon, spektrum UV, spektrum w podczerwieni, IR