Theoret. chim. Acta (Berl.) 15, 205—210 (1969)

Tautomerism in Isomeric Oxypurines*

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Received March 25, 1969

Starting with the assumption, based upon infrared spectroscopy evidence, that the oxypurines exist essentially in the keto form, calculations are performed by the CNDO/2 and the SCF MO CI methods in order to determine the most stable tautomers of these molecules with respect to the possible sites of attachement of the protons upon available ring nitrogens, and in order to evaluate their principal electronic properties. The calculations predict correctly the most stable tautomers, account satisfactorily for the observed ultraviolet absorption spectra and indicate that dipole moment measurements may be a particularly useful tool for the identification of the tautomers.

Mit der von der UV-Spektroskopie gestützten Annahme, daß die Oxypurine in der Keto-Form vorliegen, werden nach der CNDO/2- und der SCF MO CI-Methode die stabilsten tautomeren Formen dieser Moleküle bezüglich der möglichen Anlagerung von Protonen an die verfügbaren Stickstoffatome im Ring und ihre prinzipiellen elektronischen Eigenschaften berechnet. Die Rechnungen geben in Übereinstimmung mit dem Experiment die stabilsten Tautomeren richtig wieder, zeigen eine gute Übereinstimmung mit den UV-Absorptionsspektren und lassen darauf schließen, daß das Dipolmoment eine zur Identifikation der Tautomeren geeignete Größe ist.

Alors que la spectroscopie ultraviolette n'est pas capable d'indiquer sans ambiguité si les oxypurines existent préférentiellement sous la forme cétonique ou énolique, la spectroscopie infrarouge décide, elle, en faveur de la forme cétonique. Partant de cette constatation on effectue des calculs CNDO/2 et SCF MO CI pour déterminer les formes tautomères les plus stables de différentes oxypurines par rapport aux sites de fixation de protons sur les azotes du cycle. Les calculs prédisent correctement dans chaque cas les tautomères les plus probables, permettent de rendre compte de leurs spectres d'absorption et montrent l'utilité des mesures des moments dipolaires pour l'identification de ces tautomères.

In a recent work Kwiatkowski [1, 2] performed Pariser-Parr-Pople type of calculations on the electronic structure of oxypurines, essentially in view to interpret their ultraviolet spectra. In these calculations he assumed that these compounds exist predominantly in their enol form, and the results of his calculations, at least for 6- and 8-oxypurine, did not seem to contradict this assumption. It is only in the case of the 2-oxy isomer that a particularly striking disagreement between theory and experiment led him to admit that this last compound may exist in the keto form. Calculations carried out for this form gave, in fact, a more satisfactory agreement with experiment [3].

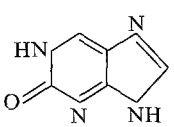
However, as it is well known, ultraviolet absorption may provide only very uncertain information about the keto-enol tautomerism in oxypurines and related compounds because of the simplicity of the spectra and their frequent similarity in the keto and enol forms [4–6]. A much more reliable method of discerning the nature of these forms is offered by infrared spectroscopy. This

* This work was sponsored by the RCP No. 173 of the Centre National de la Recherche-Scientifique and grant No. 67-00-532 of the Délégation Générale à la Recherche Scientifique et Technique.

unambiguously shows [3] that *the three isomeric oxypurines all exist essentially, both in the solid state and in solution, in the keto form,* as they all present the characteristic C=O stretching vibration (near 1670 cm^{-1} in the 2- and 6-oxypurines and near 1740 cm^{-1} in the 8-oxy isomer, and show no band which could be attributed to the O–H group.

This point granted there remains the problem of the detailed structure of the most probable keto form for each isomer. Thus, even when considering the possible keto forms for 2-oxypurine, Kwiatkowski [3] limited his investigation to the two forms, I and II, in which the hydroxyl proton is attached to N_1 or N_3 of the pyrididine ring but in which the proton of the imidazole ring is fixed at N_9. He neglected thus the possibility of the supplementary N(7)H tautomers, III and IV, which, in fact, seem to represent quite an essential aspect of the tautomerism in purines in general and in oxypurines in particular [7–11]. In this respect we may turn again for significant evidence to infra-red spectroscopy which permits through the study of the N–H stretching vibrations to distinguish between *o*-quinonoid (ν_{NH} near 3350 cm^{-1}) and *p*-quinonoid (ν_{NH} near 3450 cm^{-1}) forms. In the case of 2-oxypurine the available data favor form IV [3].

Assuming therefore on the basis of the prequoted evidence that the preferential configuration of oxypurines is the keto one (a conclusion which agrees moreover with a preliminary theoretical investigation of the problem [12] and which shall be rediscussed in more details separately) we have centered our attention on the study of the precise structure of these isomers i.e. essentially on the most probable location of the protons on the available ring nitrogens. In the case of 2-oxypurine our study thus involved the tautomeric forms I–IV and even for the sake of completeness the form V. We also performed a similar investigation for 6-oxypurine (hypoxanthine) for which we considered the tautomeric forms VI–IX and 8-oxypurine, for which we considered the tautomeric forms X–XII. Experimental evidence favors for the last two isomers the tautomeric forms VII and X, respectively.



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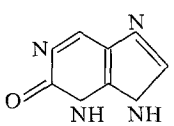

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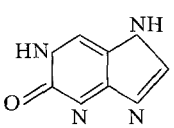

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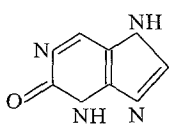

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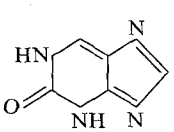

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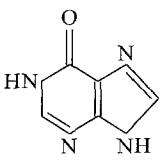
The calculations have been performed in the first place by the CNDO/2 method [13] as applied to a series of biological purines and pyrimidines recently in our laboratory [14–18]. We have been interested primarily in the total molecular energies, which determine the relative intrinsic stabilities of the different tautomers and in their dipole moments, the knowledge of which may frequently constitute, as we have suggested earlier [8, 11, 12] an excellent tool for establishing the identity of the forms. These calculations have been completed by SCF MO CI ones, as applied also in our laboratory previously to biological purines and pyrimidines [16, 19] for the sake of evaluating transition energies, these types of calculation being more suitable, in the present state of the development of the methods, for such a task than the CNDO ones.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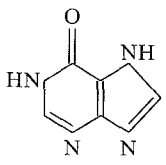
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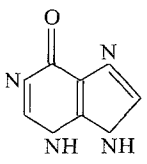
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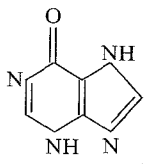
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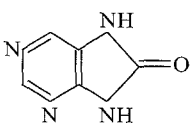
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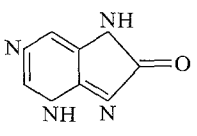
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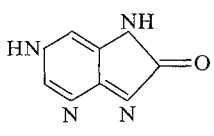
XII

The results of the calculations are presented in the Table. They indicate that:

1) In complete agreement with the deductions from infra-red data, the calculations estimate the tautomers IV, VII and X as the most stable forms of 2-oxypurine, 6-oxypurine and 8-oxypurine respectively, followed very closely by the tautomers I and VI for the first two isomers. On a relative scale the most stable of the three isomers should be the 8-oxy one (certainly because of the high content of its π-electronic delocalization), which should in fact be appreciably more stable than the 2- or 6-isomers, predicted, those, to be of comparable stability.

2) In all three isomers the most stable tautomeric form involves thus one proton at N_7, the second one being at N_3 in 2-oxypurine, at N_1 in 6-oxypurine and at N_9 in 8-oxypurine. The preferential attachement of a proton at N_7 of these isomers seems thus a general feature of their structure. Although this situation agrees with the probable preeminence of the N(7)H tautomers in solution, it should not be considered nevertheless as predjudging about the nature of the tautomer present in the crystal of these substances, because of the important

Table. *Electronic characteristics of oxypurines*

Compound		Tautomer	SCF CI method								CNDO method				
			μ (D)	θ[a]	HOMO (eV)	LEMO (eV)	S_1 theor. (mμ)	S_1[b] exp. (mμ)	S_2 theor. (mμ)	S_2[b] exp. (mμ)	μ (D)	θ[a]	HOMO (eV)	LEMO (eV)	Total energy K/mole
2-oxypurine	I	N(1)H–N(9)H	4.4	113	−8.3	0.3	317		239		5.0	119	−10.1	1.9	−64962
	II	N(3)H–N(9)H	8.0	62	−8.2	0.6	308		234		8.8	61	−10.1	2.4	−64958
	III	N(1)H–N(7)H	9.1	153	−8.3	0.1	327		237		10.9	154	−10.2	1.6	−64960
	IV	N(3)H–N(7)H	8.4	122	−8.1	0.3	339	315 [21]	230	238 [21]	9.1	119	−10.1	2.0	−64963
	V	N(1)H–N(3)H	2.0	−163	−9.3	0.0	292		252		2.3	−140	−11.2	1.5	−64959
6-oxypurine	VI	N(1)H–N(9)H	5.6	− 16	−8.0	1.0	294		240		5.9	− 16	− 9.7	2.9	−64962
(hypoxanthine)	VII	N(1)H–N(7)H	2.6	−157	−8.2	1.2	277	280 [22]	245	249 [22]	2.6	−156	−10.1	3.0	−64963
	VIII	N(3)H–N(9)H	10.5	11	−8.1	1.2	273		224		11.8	11	−10.1	3.2	−64948
	IX	N(3)H–N(7)H	3.6	41	−8.2	1.1	267		238		4.9	36	−10.2	3.1	−64955
8-oxypurine	X	N(7)H–N(9)H	2.3	−118	−8.7	0.8	264	277	227	235 [21]	1.4	−129	−11.0	3.2	−65001
	XI	N(3)H–N(7)H	4.8	− 93	−8.2	0.1	306		263		5.7	− 94	−10.1	1.7	−64960
	XII	N(1)H–N(7)H	9.4	−128	−8.1	0.2	288		281		11.2	−127	−10.0	1.8	−64956

[a] Counterclokwise with respect to the C_4–C_5 axis.

[b] The experimental absorption maxima are listed along the lines corresponding to the most stable tautomer of each oxypurine. In the case of 2- and 6-oxypurines for which there exist two tautomers of very close total energies they may represent the absorption of a mixture of such tautomers.

role of crystal packing forces in this last case. We have discussed this problem recently in some details in connection with the crystal structure of purine itself [9]. As a matter of fact, it seems that the crystal of hypoxanthine involves the N(1)H–N(9)H tautomer [20], a result which, if confirmed, could possibly be ascribed partially to the afore-mentioned environmental factors and in particular to the intermolecular interactions between hydrogen bonded and stacked hypoxanthines.

3) No general rule seems to exist about the relative values of the dipole moments with respect to the intrinsic stabilities of the different tautomers. Thus, the most stable tautomers are predicted to have a relatively low dipole moment in 6- and 8-oxypurines (1–3 *D*) and a relatively high one (≈ 9 *D*) in 2-oxypurine. The possible utility of dipole moment measurements for distinguishing between different tautomeric forms, in particular between the N(7)H and N(9)H tautomers, frequently difficult to distinguish by other physicochemical techniques, is worthwhile stressing again. On the other hand, it may also be useful to remind the reader about the limited significance of molecular dipole moments for the estimation of the relative intermolecular interaction energies (whether on hydrogen bonding or upon stacking) because of the necessity of utilizing the "monopole" approximation in such evaluations [9, 12, 19].

4) As concerns the ultraviolet absorption spectra of the three tautomers, theory and experiment agree in considering 2-oxypurine as absorbing towards the longest wavelength, 8-oxypurine as absorbing towards the shortest wavelength and 6-oxypurine as having an intermediate absorption, provided that the 280 mμ shoulder (overlooked by Kwiatkowski) observed in the absorption spectrum of hypoxanthine be recognized as a separate transition. (In fact, following Kleinwächter *et al.* [23], in dioxane solvent there are even a few shoulders in that region, the one of the longest wavelength lying at 291 mμ.) The calculations, which predict the transition of longest wavelength to lie between 277 and 294 mμ for the most probable tautomeric forms of hypoxanthine, greatly favor this viewpoint.

The agreement between theory and experiment extends also to the second absorption band in these compounds: this, following experimental evidence, lies at the longest wavelength in 6-oxypurine, at the shortest wavelength in 8-oxypurine and at intermediate wavelength in 2-oxypurine. The calculations reproduce this ordering and numerically agree within 10 mμ with the observed transitions.

It appears therefore evident that calculations based on the keto forms of oxypurines are susceptible of agreement with an extensive amount of experimental data concerning these compounds and offer therefore a complementary support for the representation of these molecules in such forms.

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