

**EXPERIMENTAL AND QUANTUM-  
CHEMICAL STUDY OF THE MECHANISM  
OF OXIDATION OF 5-HYDROXY-6-METHYL-  
URACIL BY MOLECULAR OXYGEN IN THE  
PRESENCE OF COPPER(II) IONS**

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*It has been discovered experimentally that 5,5,6-trihydroxy-6-methylpyrimidine-2,4-dione is formed on oxidizing 5-hydroxy-6-methyluracil with molecular oxygen in aqueous medium in the presence of copper(II) chloride. Ab initio and DFT calculations on the 6-31G\* basis, both in the gas phase and allowing for solvent, showed that the process proceeds with the direct participation of an activated oxygen molecule on the complex  $CuCl_2 \cdot (5\text{-hydroxy-6-methyluracil})_2$ .*

**Keywords:** 5-hydroxy-6-methyluracil, active forms of oxygen, oxidation with molecular oxygen, quantum-chemical calculations.

It is known that on fixing and activating molecular oxygen on transition metal complexes a mild and selective oxidative conversion of substrate occurs [1, 2]. On using compounds of copper as metal of variable valency the latter may be considered as models of a copper-containing oxidase. In several cases the activated oxygen molecule acts as a hydroxylating agent in relation to the ligand [3, 4].

An example of such a series of processes is the previously discovered oxidation in aqueous medium of 5-hydroxy-6-methyluracil (**1**) by molecular oxygen in the presence of copper(II) salts with the formation of 5,5,6-trihydroxy-6-methylpyrimidine-2,4-dione (**2**) [5].

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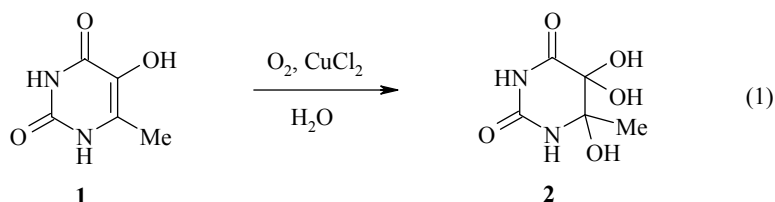
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The present work is devoted to an experimental and quantum-chemical study of the mechanism of this process.



The oxidation of uracil **1** was carried out in a thermostatted reactor at 45°C in aqueous medium (pH 3.5-4.0) at a reactant ratio of **1**-CuCl<sub>2</sub>·2H<sub>2</sub>O of 2:1 during 4 h. The course of the reaction was followed spectrophotometrically by the reduction in intensity of the absorption band at λ 278 nm (gradual saturation of the C(5)=C(6) double bond of compound **1**, Fig. 1) and by HPLC for the accumulation of compound **2** and consumption of the initial compound **1**. At the end of the process compound **2** was isolated and characterized by spectral methods and X-ray structural analysis.

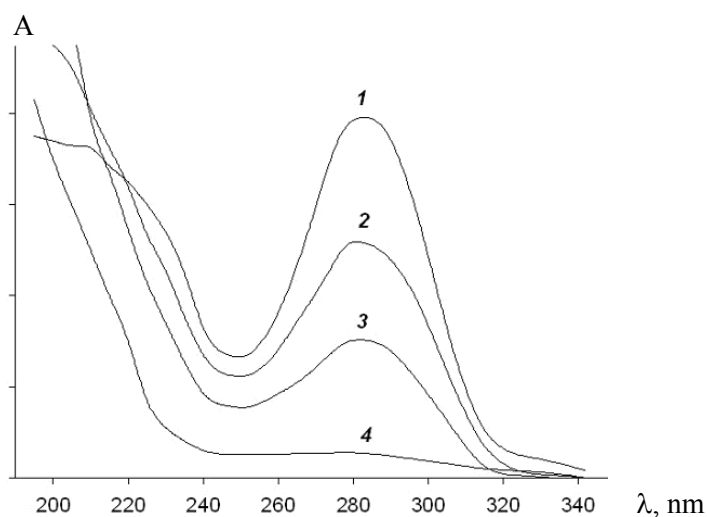
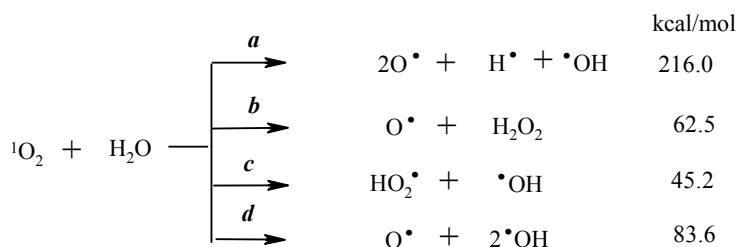


Fig. 1. Dependence of the UV spectrum of compound **1** (in water) on time of conducting the reaction (45°C), 1 – 0 min; 2 – 50 min; 3 – 150 min; 4 – 240 min.

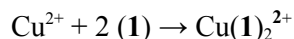
To study the probable mechanism of hydroxylation of compound **1** we undertook *ab initio* HF/6-31G\* and ROHF/6-31G\* calculations and DFT calculations on the B3LYP/6-31G\* basis, for the gas phase and allowing for solvent (water), for the separate steps (the DFT calculation was recently used effectively for calculations on the mechanism of oxidation of guanine by radicals OY, where Y = H, Me, OH [6]).

In the first step of the work we calculated the possible routes (*a-d*) of forming reactive forms of oxygen in the absence of copper salts (energy effects are indicated in kcal/mol).

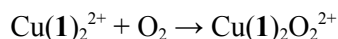


As expected, all the gas-phase reactions were endothermic. At the same time reaction *c*, which consumes the least energy, is in fact the homolysis of water at the OH bond under the action of molecular oxygen. Since we have confirmed spectrophotometrically the formation of complexes of copper(II) with 5-hydroxy-6-methyluracil of composition  $\text{Cu}(\mathbf{1})_2^{2+}$ , the following route is the most probable for the formation of reactive forms of oxygen.

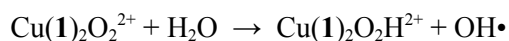
Initially complex formation of copper(II) with uracil **1** occurs.



Then complex formation of  $\text{O}_2$  with  $\text{Cu}(\mathbf{1})_2^{2+}$  takes place with the formation of adduct  $\text{Cu}(\mathbf{1})_2\text{O}_2^{2+}$ :



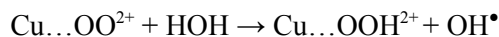
and subsequent homolysis of a water molecule with the formation of hydroxyl radicals.



These very processes, taking place either sequentially or synchronously in the supramolecular complex  $\text{Cu}(\mathbf{1})_2\text{O}_2^{2+}\cdot\text{H}_2\text{O}$ , are also evidently the most important sources of hydroxyl radicals in the reaction mixture.

If stronger ligands than compound **1** (for example trilon B) are present in the reaction medium, then oxidation of uracil does not occur.

We have carried out calculations of the energy effects of the gas-phase reaction modeling the interaction in the  $\text{Cu}(\mathbf{1})_2\text{O}_2^{2+}$  complex.



The endothermicity of the latter reaction, established by calculations (energy effect equal to 20.6 kcal/mol), is evidently overcome under actual conditions of carrying it out in a copper-containing hexacoordinated complex [7]. Consequently we chose hydroxyl radicals in the calculations as the most probable reactants for the **1**  $\rightarrow$  **2** reaction. However the formation of intermediate **3** may also be proposed [7]. Then the most probable routes of transition from uracil **1** to triol **2** may represent competing attack of the substrate by atomic (molecular) oxygen or hydroxyl radicals.

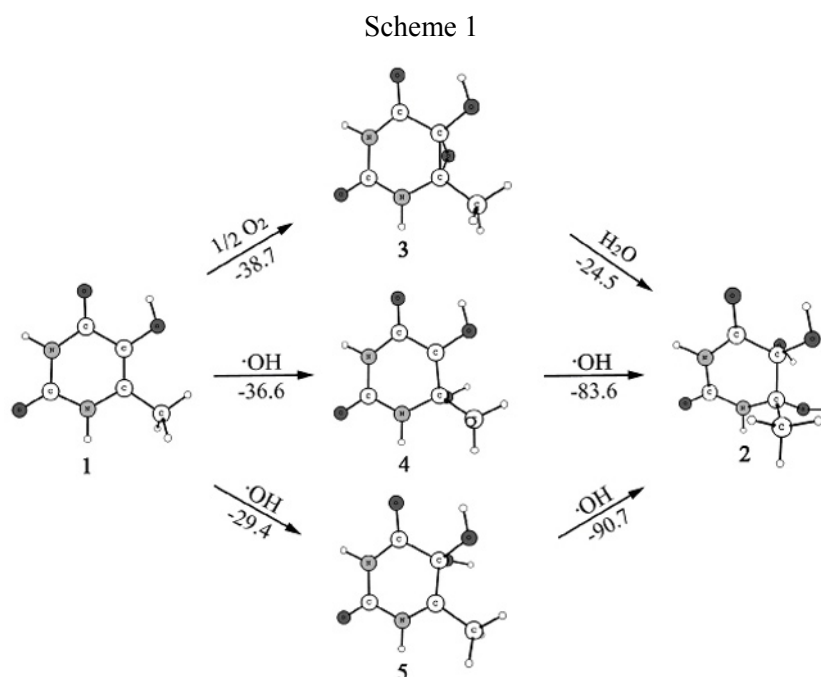


TABLE 1. Total Energies of All Structures Participating in the Conversion of **1** → **2**, Calculated by the HF/6-31G\* and B3LYP/6-31G\* Methods (numbering of structures is indicated in Fig. 2). All Radical Structures Were Calculated *ab initio* on the ROHF/6-31G\* Basis

Structure	$E_{\text{tot}}$ , a. e.		
	B3LYP/6-31G*	HF/6-31G*	HF/6-31G*+PCM
<b>1</b>	-529.35519	-526.36472	-526.38771
<b>2</b>	-680.99372	-677.25364	-677.29124
<b>3</b>	-604.54565	-601.19981	-601.23075
<b>4</b>	-605.13696	-601.77626	-601.81563
<b>5</b>	-605.12558	-601.77013	-601.80410
<b>6</b>	-680.92539	—	—
<sup>1</sup> O <sub>2</sub>	-150.25742	-149.53299	-149.53162
H <sub>2</sub> O	-76.40895	-76.01075	-76.02142
OH•	-75.72345	-75.38228	-75.39077
O•	-75.06062	-74.65660	-74.65672
HO <sub>2</sub> •	-150.89915	-150.17053	-150.18399
H <sub>2</sub> O <sub>2</sub>	-151.53321	-150.76479	-150.78520

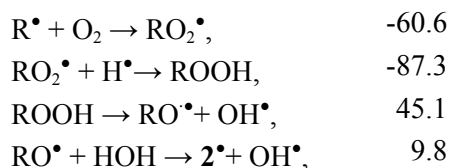
The thermal effects indicated in Scheme 1 for all the stages of the possible processes calculated both by *ab initio* methods and by the DFT method point to their exothermicity, and to routes **1** → **4** → **2** and **1** → **5** → **2** competing with one another under the reaction conditions of obtaining triol **2** from uracil **1** (the DFT and *ab initio* calculations lead to a consistent result, see Table 1).

Geometric characteristics calculated by us and obtained from X-ray structural analysis [7] are given in Fig. 2 for uracil **1**, triol **2**, and other possible participants in the transition **1** → **2**. Their energy characteristics are given in Table 1.

We think that atomic oxygen present in the system is consumed predominantly in the exothermic reaction of obtaining oxide **3**. On further hydration of it in the initial stage a water molecule approaches the bridge oxygen atom. A process then occurs of synchronous protonation of oxygen and hydroxylation of a carbon atom of the heterocycle. Hydration proceeds through the sole transition state **6** (Fig. 2), lying above the reactants in the energy scale by 18.3 kcal/mol (Fig. 3).

The stage of hydrating intermediate **3**, although also exothermic, is far less advantageous for obtaining the triol than direct barrier-free attacks by OH radicals on systems **1**, **4**, and **5**. The results of our calculations on the minimum energy route for stages **3** → **2** indicate this.

In the work we also studied another possibility of forming triol **2**, viz. through intermediate hydroperoxides formed from compounds **4** and **5**. For compounds **5** the calculated values of the thermal effects, in kcal/mol, were



Now, the oxidation of 5-hydroxy-6-methyluracil (**1**) in the presence of copper(II) chloride in aqueous medium occurs by the direct participation of an activated oxygen molecule on the CuCl<sub>2</sub>·(**1**)<sub>2</sub> complex and is completed by the formation of 5,5,6-trihydroxy-6-methylpyrimidine-2,4-dione (**2**). The formation of the triol occurs as the result of attack at the C=C double bond of uracil **1** by hydroxyl radicals. In addition to the radical route triol **2** may be obtained through the hydration of oxide **3**.

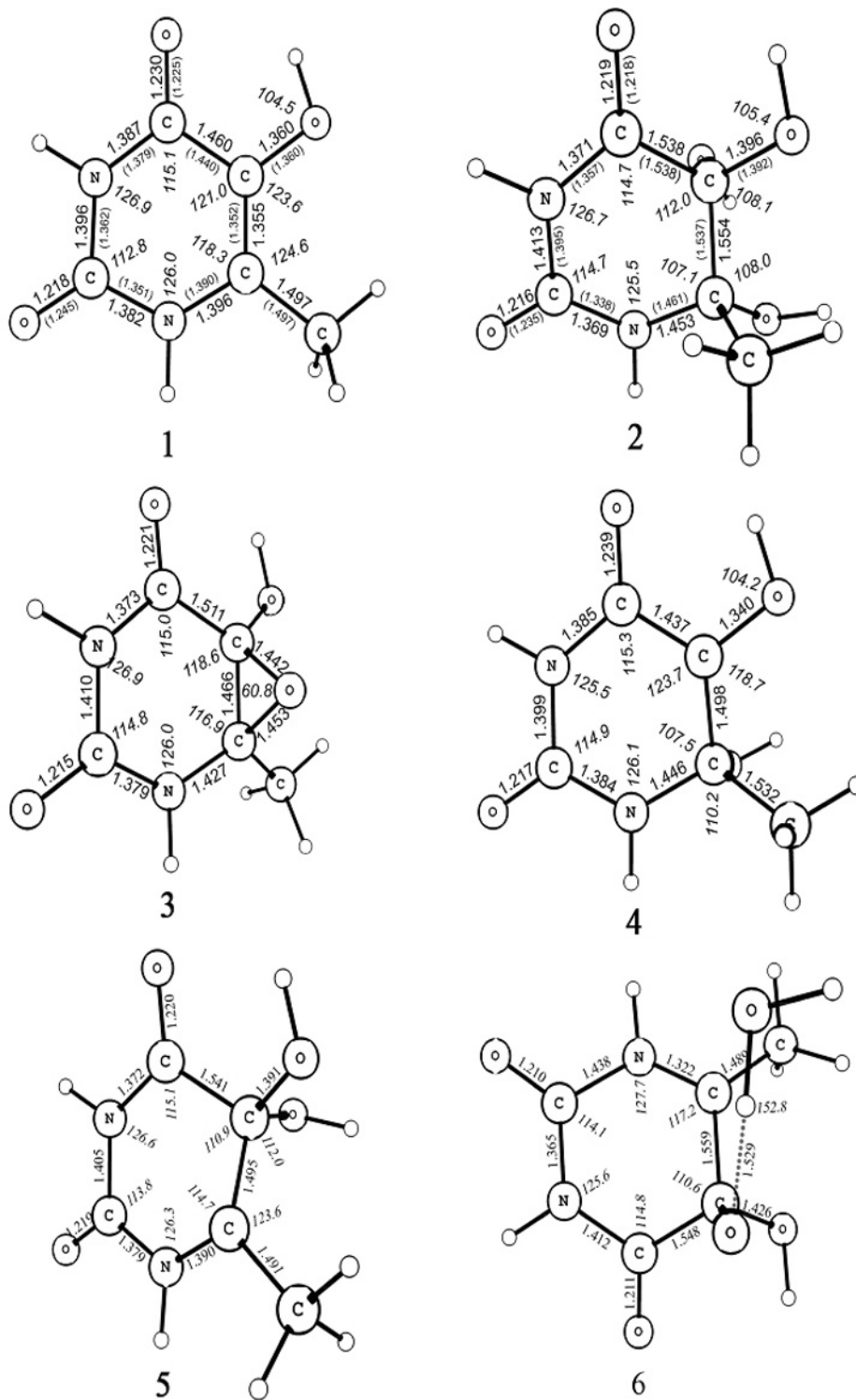


Fig. 2. Most important geometric characteristics of structures **1-5**, according to data of B3LYP/6-31G\* calculations and X-ray structural analysis (numbers in parentheses in structures **1, 2**). Also given are the most important geometric characteristics of transition state **6**. Bond lengths in Å, valence angles (italics), deg.

The obtained results correspond completely with the radical mechanism established in [8] for the formation of a glycol on UV irradiation of an aqueous solution of uracil saturated with O<sub>2</sub> in the presence of TiO<sub>2</sub>.

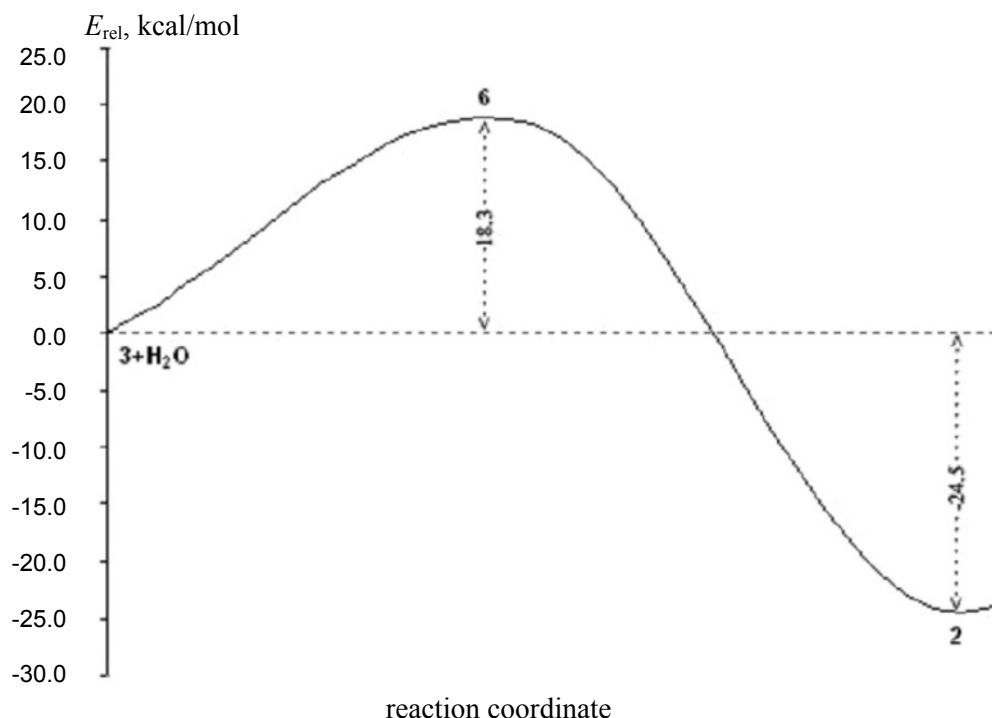


Fig. 3. Minimum energy route for the **3** → **2** reaction according to data of B3LYP/6-31G\* calculations. The relative values of energy are given in kcal/mol. From the beginning readings are taken of the sum of the total energies of the system **3** + H<sub>2</sub>O. Numbering of the structures corresponds to Fig. 2.

## EXPERIMENTAL

The UV spectra were recorded on a Specord M40 spectrometer. The <sup>13</sup>C NMR spectrum was recorded on a Bruker AM-300 impulse spectrometer (75 MHz) in D<sub>2</sub>O, internal standard was sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). The chromatographic study of the oxidation of compound **1** was carried out on a Shimadzu LC-20 AD liquid chromatograph using a column of Luna C18 phase 5 μm, of dimensions 250×4.6 cm (Phenomenex, USA). Water–acetonitrile, 95:5, was used as mobile phase at a flow rate of 1 ml/min. Detection was carried out at a wavelength of 215 nm.

5-Hydroxy-6-methyluracil (**1**), synthesized according to [9], and recrystallized from water, and "chemically pure" copper(II) chloride dihydrate were used in the work. The content of the main substance in **1** was not less than 97% [10]. Bidistilled water was used for preparing solutions.

**5,5,6-Trihydroxy-6-methylpyrimidine-2,4-dione (2).** Copper(II) chloride dihydrate (0.31 g, 1.8 mmol) was added to a solution of compound **1** (0.51 g, 3.6 mmol) in water (50 ml), and the mixture was stirred for 4 h at 45°C at atmospheric pressure. Crystals of compound **2** precipitated on slow evaporation of water from the reaction mixture. Yield was 0.54 g (85%), mp 120-121°C (water). <sup>13</sup>C NMR spectrum, δ, ppm: 156.01 (C-2); 173.97 (C-4); 84.83 (C-5); 92.19 (C-6); 20.98 (CH<sub>3</sub>). Mass spectrum (negative ions of resonance capture of electrons, 0.2-0.9 eV), *m/z* (*I*<sub>rel.</sub>, %): 158 [M-H<sub>2</sub>O]<sup>+</sup> (44), 157 [M-H<sub>3</sub>O]<sup>+</sup> (1.32), 141 (10.8), 140 (74.36), 115 [158-NCOH]<sup>+</sup> (100), 42 [CNO]<sup>+</sup> (12.1). Found, %: C 33.7; H 4.4; N 15.4. C<sub>5</sub>H<sub>8</sub>N<sub>2</sub>O<sub>5</sub>. Calculated, %: C 34.1; H 4.6; N 15.9.

**Procedure for Quantum-Chemical Calculations.** Calculations according to the theory of density functional (DFT) were carried out using the B3LYP-exchange-correlationfunctional [11, 12] and a standard basis set 6-31G\* [13]. *Ab initio* HF calculations on the 6-31G\* basis were also carried out for the majority of structures. Optimization of geometry was carried out by the analytical calculation of gradients according to the Berni scheme.

The nature of the stationary points was established on the basis of data of a calculation of normal vibrations (Hess matrix of force constants). The minimum energy route of reactions was obtained with the aid of the gradient slope from transition states in the direct and reverse direction of the transition vector.

The effect of solvent (with complete optimization of the geometry of all stationary points) was calculated within the framework of a polarized continuum model (PCM) [14-17]. Water was chosen as solvent. All calculations were carried out using the Gaussian 03 set of programs [18].

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