ISSN 0036-0244, Russian Journal of Physical Chemistry A, 2011, Vol. 85, No. 8, pp. 1358–1362. © Pleiades Publishing, Ltd., 2011.
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CHEMICAL KINETICS AND CATALYSIS

Oxidation of 1,3,7-Trimethylxanthine by Hypochlorite Ion

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Received December 22, 2010

Abstract—The kinetics of the oxidative conversion of 1,3,7-trimethylxanthine upon treatment with hypochlorite ions (OCl^-) in aqueous medium at 283–298 K and pH 8.2 was studied. The reaction order with respect to each component was determined and proved to be 1. It was established that the temperature dependence of the reaction rate follows the Arrhenius equation. The activation parameters of the reaction were measured: $E_a = 33.58$ kJ/mol, $\Delta H^{\neq} = 31.12$ kJ/mol, $\Delta S^{\neq} = -170.02$ J/(K mol), $\Delta G^{\neq} = 81.45$ kJ/mol. The stoichiometry of the reaction was studied, and the chemistry of the oxidative conversion of caffeine treated with OCl^- is discussed.

Keywords: kinetics, the Arrhenius equation, stoichiometry of reaction, oxidation, caffeine, hypochlorite ions.
DOI: 10.1134/S0036024411080152

INTRODUCTION

This work continues our series of studies on the chemical transformations of biologically active substances treated with oxidating agents containing the highly active hypochlorite group [1–8].

Caffeine (CF, 1,3,7-trimethylxanthine) is a natural dihydroxypurine alkaloid (a derivative of purine) contained in many popular and widely consumed beverages such as coffee, tea, and various energy drinks. Due to the huge popularity of caffeine-containing drinks and drugs, caffeine and its derivatives are the objects of numerous investigations. In [9], it was proved that caffeine effectively traps oxygen's free radicals; in [10], its antioxidative activity was detected in situ in instant and real coffee. At the same time, caffeine and its related compounds, which have been used in medicine to treat various pathologies for more than 100 years, are of interest to researchers due to a recent study by British biochemists [11] who reported these alkaloids form a putative basis for the development of new pharmaceuticals against cancer, cardiovascular and inflammatory diseases.

Of particular interest in this context is the study of oxidative transformations of purine derivatives in order to understand and model the metabolism of this group of biologically active substances in vitro and in vivo, and the development of methods for analyzing these compounds in different objects, medical and biological included. Research in this direction has been conducted for several decades, but the reported data on the oxidative conversion of CF were obtained under differing experimental conditions [12–21] and the available results are inconsistent.

In [12], the oxidation of CF and related methylxanthines by a free radical in Fe^{3+}–EDTA/H_2O_2/ascorbate ion systems and the Fe^{3+}–EDTA/H_2O_2/a mixture of polyphenols was discussed. It was pointed out that oxidation begins with the C8 position in the purine structure to form a hydroxy group. Further oxidation of CF produces N1-, N3-, and N7-demethylated xanthines and small amounts of 6-amino-5-(N-formylmethylamino)-1,3-dimethyluracil.

The oxidation of CF in the systems UV/H_2O_2, TiO_2/UV and in Fenton's system was studied in [13]. It was noted that CF is initially oxidized to N,N'-dimethylparabanic acid, presumably via the addition of an OH-group to the C5=C6 double bond, the second intermediate being di(N-hydroxymethyl)parabanic acid.

CF transformations during ozonation were reported in [14]. The ozonation of CF in water was performed at different pH values, including acidic conditions. Most products resulted from the imidazole ring opening upon a break of the N9=C8 double bond.

The photooxidation of CF upon treatment with peroxydiphosphate anions was studied in [15]. The major oxidation product was 1,3,7-trimethyluric acid.

Jayaram and Mayanna investigated the oxidation of CF on treatment with chloramine-B in HCl medium at 303–323 K [17] and upon treatment with chloramine-T in HCl and NaOH solutions. By studying the oxidation of CF, the authors hoped to understand the metabolism processes and to find an analogy to the processes that occurring in an organism, but this analogy was not clearly indicated in the paper. It was

reported that CF in both cases was oxidized to dimethylalloxan and methylurea.

We earlier investigated the reactivity of purine derivatives toward the oxidative effect of chloramine-B in a hydrochloric acid medium. It was established that unlike theophylline, CF yielded no colored product with chloramine-B under analogous conditions. The kinetics and thermodynamics of the oxidative reaction of chloramine-B [1, 2] with theophylline were examined, resulting in the development of highly sensitive and straightforward methods for the analytical control of theophylline in medications and body fluids that were later patented [19–21].

An analysis of recent scientific works shows that the issues of the oxidative conversions of purine derivatives including CF and theophylline are complex and ambiguous rather than simple, and the corresponding outcomes depend on the nature of the substrate (its structural analogue), and on the oxidants and experimental conditions.

It is of practical and scientific interest to study the kinetics of the oxidative transformation of CF compared to theophylline in connection with their metabolism and the developments of methods for determination of the abovementioned compounds and their structural analogs in various objects, including pharmaceuticals and biological materials.

The present work investigated the kinetics of the interaction of CF with the hypochlorite ions (OCl^-). The results of this investigation can be employed for further examination of the kinetic behavior of other structural analogs, purinic bases, nucleosides and nucleotides, and for the optimization of the corresponding analytical reactions and methods.

EXPERIMENTAL

The reagents used in this work were all of chemically pure grade. Solutions were prepared from bidistilled water. Sodium hypochlorite (HC), prepared by passing gaseous chlorine through an aqueous solution of NaOH at 273 K, was used as the oxidative reactant. The solution was stored in a light-shielded flask in order to prevent its photochemical decomposition. The prepared solution was then standardized iodometrically using a standard solution of sodium thiosulfate as the titrant and starch as the indicator. Under these conditions, the HC concentration remained constant for some time. CF (99%) was obtained from Sigma. The 0.001 M CF standard solution was prepared by dissolving an accurately weighed CF sample in bidistilled water. Its concentration was determined by UV spectroscopy at $\lambda = 273$ nm ($A_1^1 = 650$) [22].

The medium pH 8.2 was maintained by a borate buffer solution. The pH was measured by an I-160 ion meter equipped with an ESL-43-07SR glass electrode and a silver chloride reference electrode. Required amounts of 1,3,7-trimethylxanthine solution, buffer solution, and water (in order to maintain a constant total volume for all the experiments) were placed in a reaction vessel and thermostated. The temperature was maintained with an accuracy of ±0.1 K. A measured amount of the oxidant solution, thermostated at the same temperature, was quickly added to the reaction mixture. The reaction was conducted with an excess of hypochlorite ions, and an excess of phenol immediately stopped the reaction. The absorbance of the reaction product was measured using an SF-60 spectrophotometer at $\lambda = 630$ nm. The reaction stoichiometry was calculated from the composition of reaction mixtures containing different initial concentrations of CF and OCl^- 24 h after the onset of the reaction at ≈296 K.

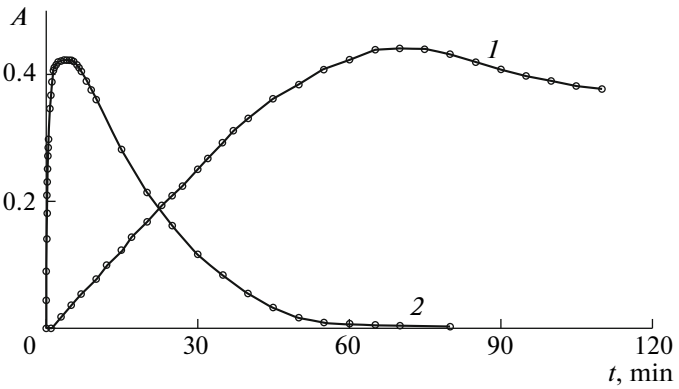
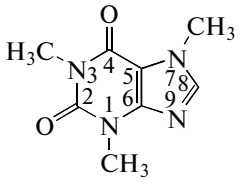


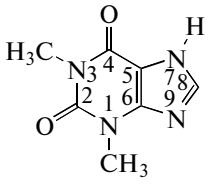
Fig. 1. Accumulation of products of the oxidation of CF (*1*) and theophylline (*2*) in one reaction medium at 296 K, pH 8.2, $[OCl^-] = 1.35 \times 10^{-2}$ M, $c_{CF} = 10.0 \times 10^{-5}$ M, $c_{theophylline} = 5.0 \times 10^{-5}$ M.

RESULTS AND DISCUSSION

The obtained results from our comparative experiments showed that in same system (mixture) the oxidation rate of theophylline is several orders of magnitude higher than that of CF treated with HC, though they are very close in terms of structural analogs and differ only in one group, a methyl radical ($-CH_3$) in the position N7.



caffeine



theophylline

Such an experimental finding in chemical kinetics (as in the given example for the oxidation of CF and theophylline by HC) proved unexpected and interesting (Fig. 1).

The results were processed on the basis of the kinetic measurements. The oxidation of CF upon

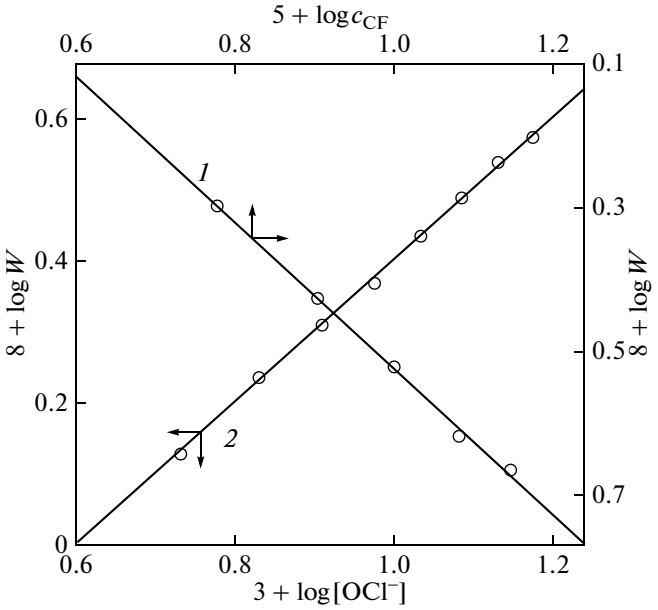


Fig. 2. Dependence of the logarithm of the rate of reaction product accumulation on the logarithm of reactant concentrations: (*1*) c_{CF}, (*2*) c_{OCl^-} at 296 K, pH 8.2.

treatment with HC under experimental conditions proceeded in such a way that kinetic curves for substrate consumption and product accumulation can be divided into two parts with different rate laws (Fig. 1). The experimental character of the kinetic curves at different CF and HC concentrations at an initial temporal segment (until 30 min) when a significant amount of CF (>70%) is converted to reaction products is linear.

From an analysis of the kinetic curves obtained for an excess of oxidant it follows that it seemingly attacks not only CF but also the formed reaction product, since a pronounced maximum of product accumulation was observed in the experiments. The product concentration began to fall after a period of some time (Fig. 1, curve *2*).

The temperature dependence of the reaction rate was studied in the range of 283–298 K. The experimental results are well described within the Arrhenius equation.

A change in the ionic strength in the reaction medium upon the addition of varied amounts of 0.1 M NaCl solution had virtually no effect on the oxidation rate. The addition of cobalt salts resulted in an increase in the reaction rate and in an increase in product yield.

The dependence of the oxidation rate on the concentration of CF and hypochlorite ions was investigated in order to determine the kinetic order of the reaction with respect to the substrate and the oxidant.

Data on the dependence of the reaction of the concentration of CF at pH 8.2, 296 K, $[OCl^-] = 1.35 \times 10^{-2}$ M is provided below.

$c_{CF} \times 10^5$, M	2.0	4.0	6.0	8.0	10.0	12.0	14.0
$W \times 10^8$, mol/(l s)	0.48	1.25	2.13	2.86	3.56	4.44	4.95

This dependence is virtually linear in the logarithmic coordinates of steady-state rate–CF concentration (Fig. 2, curve *1*).

Data on the dependence of the reaction rate on $[OCl^-]$ at $c_{CF} = 10.0 \times 10^{-5}$ M, pH 8.2, 296 K is presented below.

$[OCl^-] \times 10^3$, M	2.7	4.1	5.4	6.8	8.1	9.5	10.8	12.2	13.5	14.9
$W \times 10^8$, mol/(l s)	0.72	1.08	1.43	1.79	2.15	2.51	2.87	3.23	3.58	3.96

This dependence is also linear in logarithmic coordinates (Fig. 2, curve *2*). The line slopes for CF and the hypochlorite ion are equal to 1 within the error limits; hence, the kinetic order in CF and OCl^- is 1.

From the above data, it follows that the kinetics of the reaction of CF with OCl^- is described by the law

$$dc/dt = kc_{CF}[OCl^-], \quad (1)$$

where c_{CF}, $[OCl^-]$ represent the concentrations of CF and $[OCl^-]$, respectively.

The rate constant values calculated by Eq. (1) remain satisfactorily constant within the experimental error over the studied range of reactant concentrations. At 296 K, $k = 2.64 \pm 0.15 \times 10^{-2}$ l/(mol s).

The temperature dependence of the reaction rate in the interval 283 to 298 K (Fig. 3) is described by the Arrhenius equation

$$k = 2.21 \times 10^4 \exp(-33.58/RT),$$

where $A = 2.21 \times 10^4$ l/(mol s); activation energy $E_a = 33.58$ kJ/mol. Parameters of the activation state are as follow: activation enthalpy $\Delta H^{\neq} = 31.12$ kJ/mol; activation entropy $\Delta S^{\neq} = -170.02$ J/(mol K); Gibbs energy $\Delta G^{\neq} = 81.45$ kJ/mol.

Negative entropy and a low pre-exponential factor are characteristic of many oxidations, complex ion–molecular processes, and the formation of intermediate complexes. The high positive values of $\Delta H^{\neq}$ and $\Delta G^{\neq}$ suggest that the transition state is highly solvated.

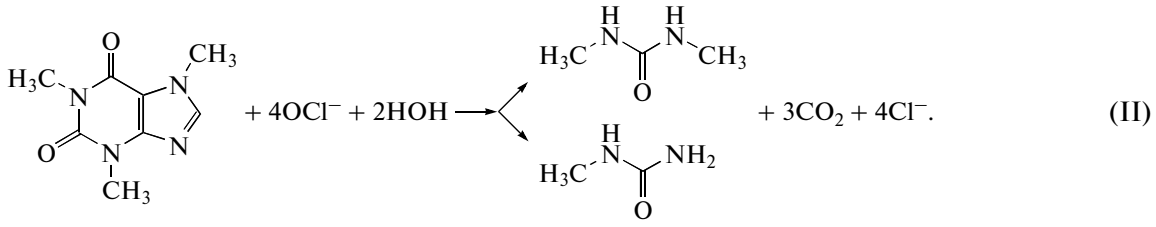
Experiments on determining the reaction stoichiometry demonstrated that the ratio of the consumed amount of OCl^- to the amount of oxidized CF ranges from 1 to 5 mol when the initial molar concentration ratio $[OCl^-]/c_{CF}$ is increased from 0.1 to 10. Oxidation stops at intermediate steps if an excess of CF is

present. Conversely, if an excess of OCl^- is present, the process proceeds to such highly oxidized end products as symmetric N,N'-dimethylurea, methylurea, and so on.

Reduction of the hypochlorite ion to the chloride ion requires the addition of two electrons and two protons. According to our experiments, 4 mol OCl^- is thus required for the deep oxidation of 1 mol CF:

$$4OCl^- + 8\bar{e} + 8H^+ = 4Cl^- + 4H_2O. \quad (I)$$

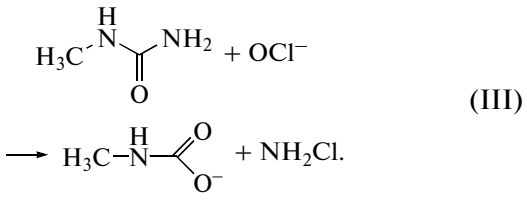
The oxidation of CF upon treatment with OCl^- is accordingly described by the equation:



(II)

Given the experimental results and our analysis of literature data on the conversions of purinic bases and xanthine derivatives [23], the mechanism of reaction (II) can be represented as follows: The primary product of the reaction of HC with CF in a weakly alkaline aqueous medium is 8-oxocaffeine, which undergoes further oxidation by OCl^- to produce a series of products of the cleavage of the heterocyclic purine system [24].

Saturation of the conjugated π electron system at the C5=C6 double bond leads to the rapid disappearance of the characteristic ultraviolet absorbance at $\lambda_{max} = 273$ nm. After the formation of 5,6-dioxocaffeine, a hydrolytic cleavage at the C5–C6 bond occurs to form methylparabanic acid. Upon further oxidation, this is decarboxylated to yield methylurea, which is in turn oxidized to release chloramine:



(III)

The formation of chloramine was detected photometrically at $\lambda_{max} = 244$ nm.

Chloramine is unstable thermodynamically, and its accumulation, chemical equilibrium, and hydrolysis can be characterized by curves *1* and *2* (Fig. 1). Figure 1 shows that there is an initial increase in the product concentration. An equilibrium plateau is then observed, after which the concentration of the reaction product begins to gradually decline.

Aqueous solutions in the reaction system under investigation contain an excess of hydroxide ions resulting from the hydrolysis of OCl^-:

$$OCl^- + HOH \leftrightarrow HOCl + OH^-. \quad (IV)$$

The thermodynamic equilibrium constant of the reaction

$$NH_2Cl + OH^- \leftrightarrow NH_2OH + Cl^- \quad (V)$$

in an alkaline medium is determined by the ratio

$$K = a_{NH_2OH}a_{Cl^-}/(a_{NH_2Cl}a_{OH^-}), \quad (2)$$

where a_{NH_2OH}, a_{Cl^-}, a_{NH_2Cl}, a_{OH^-} are the activities of the corresponding particles.

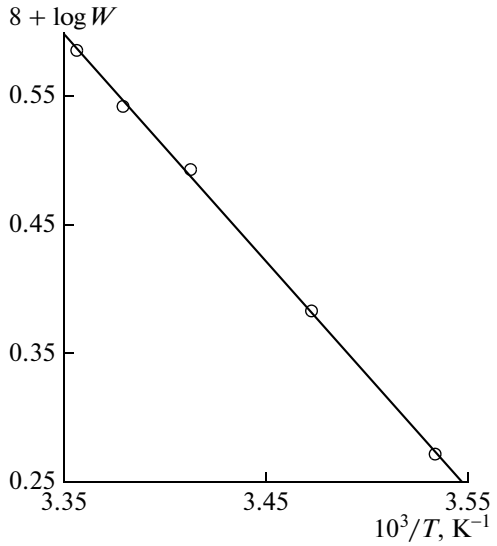


Fig. 3. Dependence of the reaction rate logarithm on the inverse temperature at $c_{OCl^-} = 1.35 \times 10^{-2}$ M, $c_{CF} = 10.0 \times 10^{-5}$ M, pH 8.2.

CONCLUSIONS

We established that the oxidative transformation of CF upon treatment with HC occurs slowly at 283–298 K in several steps. The formation and composition of intermediate and final products depend on the substrate/oxidant molar ratio, their nature, and the reaction conditions. It would be interesting to continue this study by investigating the kinetics and mechanism of the oxidative conversion of other purine derivatives, including nucleosides and nucleotides.

A method for determining CF in medico-pharmaceutical objects has been developed on the basis of these investigations into the kinetics of the CF oxidation. This material has been submitted to the Belarus National Center for Intellectual Property.

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