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Quercetinase model studies. The oxygenation of flavonol catalyzed by a cationic 2,2′-bipyridine copper(II) flavonolate complex [1]

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Abstract

The catalyst $[Cu^{II}(bpy)(fla)]ClO_4$, **1**, was prepared from $Cu(CH_3CN)_4ClO_4$, 2,2′-bipyridine (bpy), flavonol (flaH), and dioxygen in acetonitrile at ambient conditions. The flavonolato copper complex **1** reacts with molecular oxygen slowly at room temperature but reasonably fast at around 100°C in acetonitrile or DMF to the *O*-benzoylsalicylato copper complex $[Cu^{II}(bpy)(O\text{-bs})]ClO_4$ in good yield. Kinetics of the oxygenation of **1** shows that there are two kinetically not interpretable segments of the reaction, however **1** is a good catalyst for the oxygenation of flavonol to *O*-benzoylsalicylic acid (*O*-bsH) at around 100°C. Kinetic studies of the catalytic reaction resulted in the rate expression $-d[flaH]/dt = k_{obs}[flaH][catalyst]^2[O_2]^2$. The reaction constant at 100°C was found to be $k_{obs} = 7.20 \pm 0.13 \times 10^{10}$ mol^{-4} dm^{12} s^{-1}, with activation parameters $\Delta H^{\ddagger} = 80.83 \pm 3.14$ kJ mol^{-1}, $\Delta S^{\ddagger} = 179.61 \pm 8.82$ J mol^{-1} K^{-1}. © 1998 Elsevier Science B.V.

Keywords: Copper; Dioxygenation; Quercetinase; Flavonolate

1. Introduction

Many enzymes utilize molecular oxygen for the biological degradation of organic substrates. These enzymes usually contain redoxactive metals such as copper and iron ions at their active center. Since oxidation reactions in biological systems as well as in the industry play an important role, the understanding of the nature of these reactions is of primary interest. Mechanistic aspects of both areas may have common basic chemistry and due to that interest in copper-containing monooxygenase [1–4] and dioxygenase enzymes [5–7] and their structural and functional model systems are still under intense investigation [8].

Aspergillus or *Pullularia* species oxidatively degrade quercetin (3′,4′,5,7-tetrahydroxyflavonol) into a depside (phenolic carboxylic acid ester) and carbon monoxide (Eq. (1)). Quercetin 2,3-dioxygenase, a copper-containing metalloenzyme, is responsible for the oxygena-

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[1] Dedicated to Professor Gottfried Huttner on the occasion of his 60th birthday.

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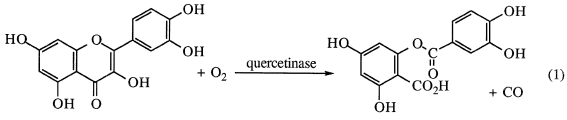
tion reaction [9–13]. Previous studies have shown that the copper is in the cupric form in the resting state of the enzyme, its ligand environment is still obscure, and quercetin coordinates probably through its 3-hydroxy and 4-carbonyl group to the copper ion [14–16].



Some model reactions on the oxygenation of quercetin and flavonol have been carried out to gain information about this curious reaction. Base catalyzed oxygenation of quercetin and related 3-hydroxyflavones under aqueous [17] and non-aqueous [17] conditions, photosensitized oxygenations [18], and reactions with superoxide ion [19] have been studied. Metal complexes of cobalt [18] and copper [20] have been found to act as catalysts for the oxygenation reaction. Copper(I) [21] and copper(II) [16] flavonolate complexes were also successfully used for the oxygenation of flavonol.

Recently we have been concerned with the synthesis of copper flavonolate complexes and their oxygenation reactions as well as using them as catalyst for the oxygenation of flavonol [16,21]. In the present paper we report details for the synthesis and characterization of $[Cu^{II}(bpy)(fla)]ClO_4$ and its oxygenated product $[Cu^{II}(bpy)(O\text{-bs})]ClO_4$, and kinetic studies on the stoichiometric oxygenation of $[Cu^{II}(bpy)(fla)]ClO_4$ and the catalytic oxygenation of flavonol catalyzed by $[Cu^{II}(bpy)(fla)]ClO_4$.

2. Experimental

2.1. Instrumentation

UV-VIS spectra were obtained on a Shimadzu UV-160A spectrophotometer. CHN analyses were performed on a Carlo Erba 1108 CHNS-O instrument. IR spectra were obtained on a Specord 75 IR (Carl Zeiss, Jena) and ESR spectra on a JEOL JES-FE/3X ESR spectrometer. GC-MS determinations were performed on a Hewlett Packard 5890 Series II gaschromatograph and magnetic measurements on a Bruker B-E 10b8 magnetic balance.

2.2. Materials

Solvents (acetonitrile, diethyl ether and DMF) were purchased from Reanal (Budapest) in analytically pure quality. Acetonitrile was refluxed on P_2O_5, distilled, then redistilled from K_2CO_3 under argon or dinitrogen. Diethyl ether was distilled from K–Na amalgam under Ar atmosphere. DMF was distilled with benzene and water (250 g DMF, 20 g benzene and 10 g water) and stored under nitrogen in the dark to avoid direct light. Purified, water- and oxygen-free solvents were stored under argon or dinitrogen. 2,2′-bipyridine was purchased from Reanal (Budapest) and used without further purification. Flavonol [22,23], $[Cu^{I}(CH_3CN)_4]ClO_4$ [24] and *O*-benzoylsalicylic acid [25] were obtained by literature methods. Gaseous oxygen from Messergriesheim was 99.60% and passed through P_2O_5 and Blaugel in order to remove traces of water and other impurities.

2.3. $[Cu^{II}(fla)(bpy)]ClO_4$

To a solution of 0.149 g (0.456 mmol) $[Cu^{I}(CH_3CN)_4]ClO_4$ in 5 cm^3 acetonitrile 0.109 g (0.455 mmol) flavonol and 0.071 g (0.456 mmol) 2,2′-bipyridine were added. The reaction mixture was stirred for 2 h at room temperature under dioxygen atmosphere. The green precipitate formed was filtered, washed several times with diethyl ether and dried to give green powder. Crystals were obtained from acetonitrile as green prisms. Yield: 0.230 g (90.76%). Anal. Found: C, 55.20; H, 2.49; N, 5.32. Calc.: C,

53.95; H, 3.06; N, 5.04%. IR (KBr): 1540s, 1485s, 1445s, 1420s, 1090s, 1055s, 755s, 1310m, 1225m, 1200m, 615m, 3045w, 900w, 875w, 570w, 480w cm^{-1}. UV-VIS (DMF): 426.5 (log ε 4.26), 312.0 (4.27), 265.5 (4.30) and 637.0 (2.30) nm. ESR (acetonitrile): $g = 2.124$, $A = 77.68$ G. Molar magnetic susceptibility: $\chi = 2.660 \times 10^{-6}$ c.g.s. unit, $\mu_{eff} = 2.06$ BM/Cu^{II}.

2.4. $[Cu^{II}(O\text{-}bs)(bpy)]ClO_4$

To a solution of 0.932 g (2.871 mmol) $[Cu^{I}(CH_3CN)_4]ClO_4$ in 30 cm^3 acetonitrile 0.695 g (2.872 mmol) *O*-benzoylsalicylic acid and 0.448 g (2.872 mmol) 2,2′-bipyridine were added. The reaction mixture was stirred for 1 h at room temperature under dioxygen atmosphere. The blue precipitate was filtered, washed several times with diethyl ether and dried to give blue powder. Yield: 0.703 g (82.01%). Anal. Found: C, 52.30; H, 3.59; N, 7.26. Calc.: C, 51.91; H, 3.33; N, 6.99%. IR (KBr): 1725s, 1600s, 1435s, 1250s, 1080s, 1560m, 1465m, 1385m, 1310m, 1020m, 760m, 700m, 620m, 3050w, 850w, 740w, 660w cm^{-1}. UV-VIS (DMF): 302.5 (log ε 4.13), 262.5 (4.00), 312.0 (4.09) and 670.0 (1.58) nm. ESR (toluene): $g = 2.138$, $A = 61.22$ G. Molar magnetic susceptibility: $\chi = 1.619 \times 10^{-6}$ c.g.s. unit, $\mu_{eff} = 1.71$ BM/Cu^{II}.

2.5. Oxygenation of $[Cu^{II}(fla)(bpy)]ClO_4$ in acetonitrile

0.011 g (0.20 mmol) $[Cu^{II}(fla)(bpy)]ClO_4$ dissolved in 5 cm^3 acetonitrile was stirred in a stainless steel pressure vessel for a week at room temperature under dioxygen at 92 bar. The solvent was then evaporated in vacuum, the residue was washed several times with diethyl ether and dried to give $[Cu^{II}(O\text{-bs})(bpy)]ClO_4$ as blue powder. Yield: 0.010 g (93.51%). The spectral and magnetic features were identical to that prepared from $[Cu^{I}(CH_3CN)_4]ClO_4$ and *O*-benzoylsalicylic acid (2.4).

2.6. Oxygenation of $[Cu^{II}(bpy)(fla)]ClO_4$ in DMF

1.362 g (2.45 mmol) $[Cu^{II}(fla)(bpy)]ClO_4$ was dissolved in 20 cm^3 DMF and refluxed for 9 h under dioxygen atmosphere. 2 cm^3 diazomethane solution (in diethyl ether) was added to 1 cm^3 of the reaction mixture at room temperature and *N,N*-dimethylbenzamide (17.90%), salicylic acid (29.06%), benzoic acid (18.39%), and *O*-benzoylsalicylic acid (23.73%) was determined by GC-MS.

2.7. Catalytic oxygenation of flavonol catalyzed by $[Cu^{II}(fla)(bpy)]ClO_4$

1.1249 g (4.72 mmol) flavonol and 0.0537 g (0.10 mmol) $[Cu^{II}(fla)(bpy)]ClO_4$ was dissolved and stirred at reflux temperature in 15 cm^3 DMF for 10 h under dioxygen atmosphere. 2 cm^3 diazomethane solution (in diethyl ether) was added to 1 cm^3 of the reaction mixture at room temperature and the amount of *N,N*-dimethylbenzamide (22.78%), salicylic acid (43.20%), benzoic acid (28.36%), and *O*-benzoylsalicylic acid (2.76%) was determined by GC-MS.

2.8. Kinetic oxygenations

Reactions were carried out at controlled temperature in a thermostated glass reactor (50 cm^3) provided with an electronically controlled magnetic stirrer connected to a manometer containing dioxygen of various pressure. The concentration of the complex $[Cu^{II}(fla)(bpy)]ClO_4$ and flavonol was followed spectrophotometrically by taking samples at regular time intervals, diluted them to appropriate concentration, and monitored at 426.5 (log $\varepsilon = 4.26$) and 342.5 (log $\varepsilon = 4.24$) nm.

3. Results and discussion

3.1. Oxygenation of $[Cu^{II}(bpy)(fla)]ClO_4$

The structure of the complex $[Cu^{II}(bpy)(fla)]ClO_4$ has been determined by X-ray crystallography, and was found to exhibit a square pyramidal geometry around the Cu^{II} ion [26]. The N-atoms of bpy and the O-atoms of the 3-OH and 4-C=O groups of flavonolate are in the basal plane, while the perchlorate O-atom occupies the apical position (Fig. 1). The oxygenation of the copper(II) flavonolate complex $[Cu^{II}(bpy)(fla)]ClO_4$ to the *O*-benzoylsalicylato copper(II) complex $[Cu^{II}(bpy)(O\text{-}bs)]ClO_4$ either in acetonitrile or DMF at room temperature and atmospheric dioxygen pressure is negligible. However if the reaction is carried out at elevated dioxygen pressure (92 bar) at room temperature in a pressure vessel for one week in acetonitrile the yield on $[Cu^{II}(bpy)(O\text{-}bs)]ClO_4$ is over 90%. In a similar fashion the oxygenation of $[Cu^{II}(bpy)(fla)]ClO_4$ in DMF at 100°C results in the *O*-benzoylsalicylato copper(II) complex in 89% yield. In both cases carbon monoxide formation contaminated with a small amount of carbon dioxide has been observed.

After establishing the stoichiometry of the reaction kinetic studies on the oxygenation of $[Cu^{II}(bpy)(fla)]ClO_4$ were carried out in DMF at 100°C and atmospheric dioxygen pressure. The reaction was monitored by electron spectroscopy at 426.5 nm. A typical oxygenation experiment can be seen in Fig. 2. Plotting the time dependence of the concentration of $[Cu^{II}(bpy)(fla)]ClO_4$ (Fig. 3) shows two segments, indicating that there are at least two processes occurring consecutively. The initial gradient hints to a slower process, while the second one may be considered as the actual oxygenation process of the coordinated flavonolate ligand. The first one is believed to correspond to a slow conversion of the complex to another one, which then reacts with dioxygen to the endproduct. A conversion of $[Cu^{II}(bpy)(fla)]ClO_4$ to $Cu^{II}(bpy)(fla)_2$ may be the case, as found with the similar complex ligated by tmeda. Here $Cu(fla)_2$ could be isolated and the formation of $[Cu(fla)_2(tmeda)]ClO_4$ was assumed on the basis of kinetic data [27]. Efforts to fit the curve or parts of it, in order to establish the reaction order with respect to the copper flavonolate complex, failed. The reason for that may lay in the complexity of the reaction, or the starting complex serves only as a

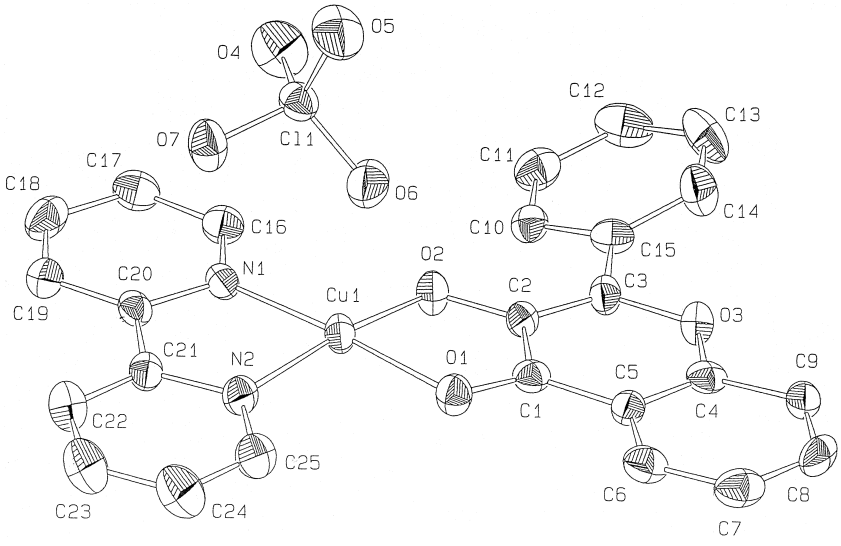


Fig. 1. The X-ray structure of $[Cu^{II}(bpy)(fla)]ClO_4$.

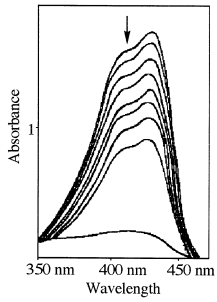


Fig. 2. Spectral changes accompanying the oxygenation of $[Cu^{II}(bpy)(fla)]ClO_4$ in DMF at 100°C and 1 bar dioxygen pressure.

precursor for other active species, giving rise to the complexity of the system.

3.2. Oxygenation of flavonol catalyzed by $[Cu^{II}(bpy)(fla)]ClO_4$

Since for the enzymatic reaction of quercetin 2,3-dioxygenase it has been assumed that quercetin binds to copper(II) through its 3-hydroxy and 4-keto groups [14–16], and structural model studies with simple copper compounds and flavonol evidenced this type of coordination [14–16] we attempted as a key model reaction the oxygenation of flavonol by the copper flavonolate complex $[Cu^{II}(bpy)(fla)]ClO_4$. The oxygenation did not proceed fast enough in acetonitrile or other solvents at room temperature as already established for the stoichiometric oxygenation reaction. In DMF however at elevated temperature the reaction progressed well, and the disappearance of the flavonol and the concentration of the catalyst could be monitored conveniently by electron spectroscopy. The spectral characteristics of a DMF solution of the $[Cu^{II}(bpy)(fla)]ClO_4$ complex and its oxygenated product have been established. The complex $[Cu^{II}(bpy)(fla)]ClO_4$ exhibits absorption at 426.5 nm. Flavonol shows absorbance at 342.5 nm [28,29]. Spectral changes accompanying addition of dioxygen to the DMF solution show that the absorption peaks at 306.5 and 343.0 nm decrease, while that at 426.5 nm increases with time and at 381.0 nm there is an isosbestic point (Fig. 4).

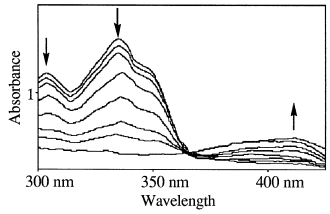


Fig. 4. Spectral changes accompanying the oxygenation of flavonol catalyzed by $[Cu^{II}(bpy)(fla)]ClO_4$ at 100°C, 1 bar dioxygen pressure and 1.15×10^{-5} mol dm^{-3} catalyst concentration.

In order to understand the reaction of the oxygenation of flavonol catalyzed by $[Cu^{II}(bpy)(fla)]ClO_4$ the kinetics of the reaction was investigated. The reaction could be followed easily by electron spectroscopy. Flavonol has absorptions in DMF at 340.0 nm and the

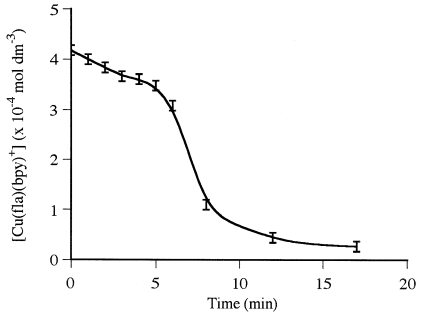


Fig. 3. Time dependence of the oxygenation of $[Cu^{II}(bpy)(fla)]ClO_4$ and excess dioxygen ($P_{O2}=1$ bar) in DMF monitored at 426.5 nm ($[Cu]_0=4.17\times10^{-4}$ mol dm^{-3}, $t=$ 100°C, $[O_2]=2.54\times10^{-3}$ mol dm^{-3}).

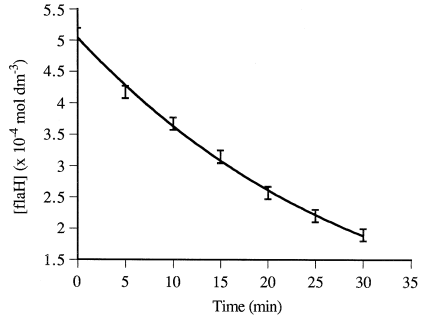


Fig. 5. Time dependence of the catalytic oxygenation of flavonol catalyzed by $[Cu^{II}(bpy)(fla)]ClO_4$ ($[flaH]_0=5.10\times10^{-4}$ mol dm^{-3}, $[Cu]=1.15\times10^{-5}$ mol dm^{-3}, $t=100$°C, $[O_2]=7.37\times10^{-3}$ mol dm^{-3}).

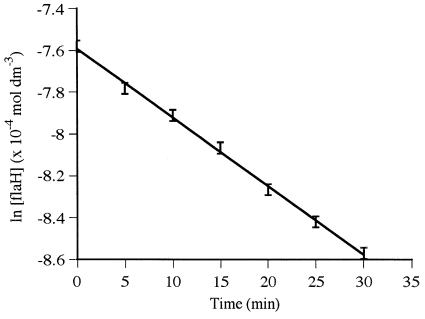


Fig. 6. The plot of the log[flaH] vs. time for the $[Cu^{II}(bpy)(fla)]ClO_4$-catalyzed oxygenation of flavonol ($[flaH]_0 = 5.10\times10^{-4}$ mol dm^{-3}, $[Cu] = 1.15\times10^{-5}$ mol dm^{-3}, $t = 100$°C, $[O_2] = 7.37\times10^{-3}$ mol dm^{-3}).

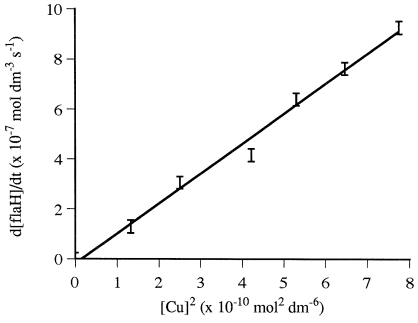


Fig. 8. Initial rate of reaction plotted against the second power of $[Cu^{II}(bpy)(fla)]ClO_4$ concentration ($t = 100$°C, $[O_2] = 7.37\times10^{-3}$ mol dm^{-3}, $[flaH]_0 = 3.25\times10^{-4}$ mol dm^{-3}).

flavonolato complex shows charge transfer bands in DMF at 426.5 nm. In the presence of a large excess of flavonol and constant dioxygen pressure the decrease of the flavonol concentration takes the course as shown in Fig. 5. Plotting the logarithm of the flavonol concentration against the time a straight line was obtained as shown in Fig. 6, indicating that the rate of the reaction is first order with respect to the substrate. The first order dependence in the substrate flavonol could also be established by plotting the initial reaction rate of the oxygenation of flavonol against the initial concentration of flavonol giving again a straight line (Fig. 7). The rate of oxygenation of flavonol at different catalyst concentration and at various dioxygen pressure (assuming the validity of Dalton's law) was also determined. Plotting the reaction rate of the oxygenation versus the catalyst concentration a parabolic curve was obtained, indicating that the reaction order with respect to the catalyst is two. Plots of the reaction rate against the square of the catalyst concentration resulted in a straight line (Fig. 8), and similarly by plotting the reaction rate against $[O_2]^2$ again a straight line was established (Fig. 9). The rate expression therefore has the form

$$d[\text{flavonol}]/dt = k_{obs}[\text{catalyst}]^2[\text{flavonol}][O_2]^2 \quad (2)$$

where [catalyst] is the initial concentration of the complex. The value of k_{obs} is calculated as $7.20 \pm 0.13 \times 10^{10}$ dm^{12} mol^{-4} s^{-1} at 100°C. Details and data of the kinetic measurements are compiled in Table 1. By measuring the reaction rate of the $[Cu^{II}(bpy)(fla)]ClO_4$-catalyzed flavonol oxygenation at temperatures of 90 and

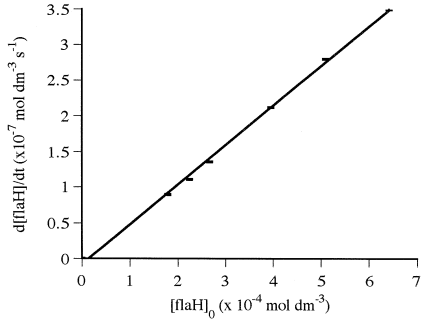


Fig. 7. Initial rate of reaction as a function of the flavonol concentration ($t = 100$°C, $[O_2] = 7.37\times10^{-3}$ mol dm^{-3}, $[Cu] = 1.15\times10^{-5}$ mol dm^{-3}).

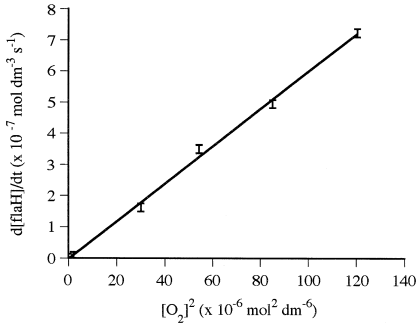


Fig. 9. Initial rate of reaction as a function of $[O_2]^2$ ($t = 100$°C, $[Cu] = 1.15\times10^{-5}$ mol dm^{-3}, $[flaH]_0 = 5.96\times10^{-4}$ mol dm^{-3}).

Table 1
Kinetic data for the oxygenation of $[Cu(bpy)(fla)]ClO_4$ and the catalytic oxygenation of flavonol catalyzed by $[Cu(bpy)(fla)]ClO_4$

Reaction No.	t (°C)	$[O_2]$ ($\times 10^{-3}$ mol dm^{-3})	[flaH] ($\times 10^{-4}$ mol dm^{-3})	d[flaH]/dt ($\times 10^{-7}$ mol dm^{-3} s^{-1})	$k[Cu]^2[O_2]^2$ ($\times 10^{-4}$ s^{-1})	$k[O_2]^2$ ($\times 10^{6}$ mol^{-2} dm^{6})	k_{obs} ($\times 10^{10}$ mol^{-4} dm^{12} s^{-1})
1	100	1.47	6.249	0.129	0.206	0.156	7.211 ± 0.119
2	100	7.37	6.416	3.497	5.450	4.114	7.569 ± 0.043
3	100	7.37	1.797	0.937	5.214	3.936	7.242 ± 0.360
4	100	7.37	2.671	1.255	4.699	3.547	6.526 ± 0.033
5	100	7.37	2.248	1.105	4.915	3.710	6.826 ± 0.123
6	100	7.37	3.956	2.116	5.349	4.038	7.429 ± 0.074
7	100	7.37	5.102	2.798	5.484	4.139	7.615 ± 0.152
8	100	7.37	2.632	1.299	4.940	3.729	6.861 ± 0.139
9	100	7.37	2.416	4.165	1.725	4.081	7.509 ± 0.229
10	100	7.37	3.080	9.246	3.004	3.873	7.126 ± 0.083
11	100	7.37	3.252	3.056	9.403	3.762	6.922 ± 0.151
12	100	7.37	2.942	6.392	2.174	4.099	7.542 ± 0.168
13	100	7.37	2.894	7.613	2.632	4.070	7.489 ± 0.091
14	90	7.23	7.445	1.774	2.383	1.799	3.442 ± 0.091
15	108	7.18	7.545	6.600	8.748	6.603	12.801 ± 0.139
16	100	9.23	5.960	4.947	8.300	6.265	7.352 ± 0.099
17	100	10.98	6.038	7.236	1.198	9.042	7.504 ± 0.060
18	100	5.481	6.314	1.621	2.567	1.938	6.451 ± 0.104

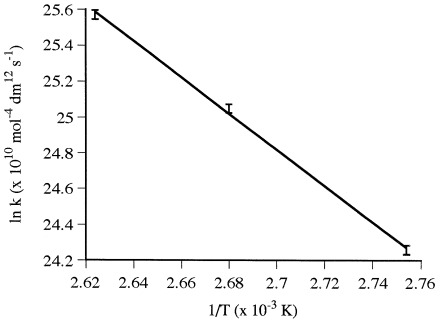
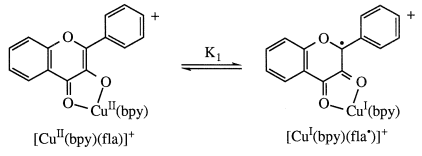


Fig. 10. Arrhenius plot for the catalytic oxygenation of flavonol catalyzed by $[Cu^{II}(bpy)(fla)]ClO_4$ ($[Cu] = 1.15\times10^{-5}$ mol dm^{-3}, $[flaH]_0 = 7.75\times10^{-4}$, $[O_2] = 1$ bar).

108°C the Arrhenius plot shown in Fig. 10 has been obtained. The activation parameters calculated from Fig. 10 for the catalytic oxygenation of flavonol gave the following values: $\Delta H^{\ddagger} = 80.83 \pm 3.14$ kJ mol^{-1} and $\Delta S^{\ddagger} = 179.61 \pm 8.82$ J mol^{-1} K^{-1}.

The high complexity of the rate equation suggests that the mechanism of the catalytic oxygenation of flavonol in the presence of $[Cu^{II}(bpy)(fla)]ClO_4$ is rather complicated compared to that of the similar *N,N,N′,N′*-tetramethylethylene (tmeda) copper complex where an overall third order rate expression could be established [27]. We suggest in the case with the bpy ligand reaction steps, which are in agreement with the kinetic data obtained. In the $[Cu^{II}(bpy)(fla)]ClO_4$ complex there may be an *intramolecular* electron transfer from the flavonolate ligand (fla) to Cu(II) yielding $[Cu^{I}(bpy)(fla^{\cdot})]ClO_4$ as shown in Eq. (3).



(3)

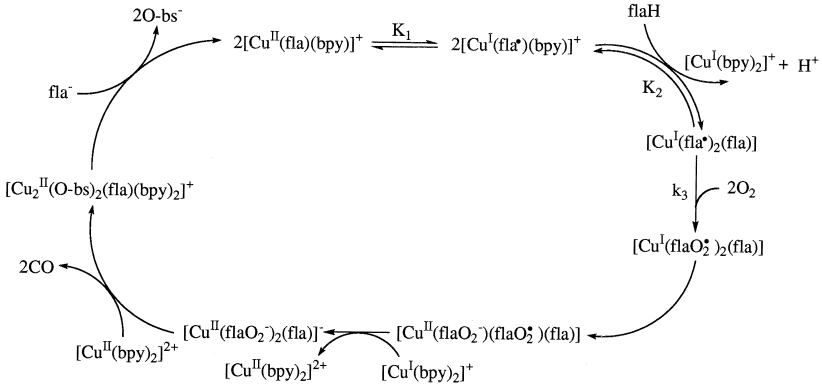
The value of K_1 is probably very small. Two moles of $[Cu^{I}(bpy)(fla^{\cdot})]ClO_4$ may react then with flavonol in a reversible step to $[Cu^{I}(fla^{\cdot})_2(fla)]$ and $[Cu^{I}(bpy)_2]^+$ as indicated in Eq. (4).

$$\left[Cu^{I}(fla^{\cdot})(bpy)\right]^+ + flaH \overset{K_2}{\rightleftharpoons} \left[Cu^{I}(fla^{\cdot})_2(fla)\right] + \left[Cu^{I}(bpy)_2\right]^+ + H^+ \quad (4)$$

The equilibrium is again shifted much to the left side with a small value for K_2. The compound $[Cu^{I}(fla^{\cdot})_2(fla)]$ is probably octahedral and $[Cu^{I}(bpy)_2]^+$ is tetrahedral as evidenced in the similar complex $[Cu^{I}(bpy)_2]_2[Cu^{I}(fla)_3]$ from which an X-ray structure is also available [30]. Among the products of equilibrium (4) $[Cu^{I}(fla^{\cdot})_2(fla)]$ has three and $[Cu^{I}(bpy)_2]^+$ one redoxactive center. All redoxactive centers may react with dioxygen, however the very clean second order dependence of the reaction rate on the dioxygen concentration makes an assumption plausible that probably $[Cu^{I}(fla^{\cdot})_2(fla)]$ reacts with two moles of dioxygen in the rate-determining step as a radical radical reaction (5) to the primary product $[Cu^{I}(flaO_2^{\cdot})(fla)]$, which is transformed in fast consecutive steps to $[Cu^{II}(bpy)(O\text{-bs})]^+$ and CO. Flavonol replaces then the *O*-benzoylsalicylato ligand again in a fast reaction.

$$\left[Cu^{I}(fla^{\cdot})_2(fla)\right] + 2O_2 \underset{\text{slow}}{\overset{k_3}{\rightarrow}} \left[Cu^{I}(flaO_2^{\cdot})_2(fla)\right] \quad (5)$$

The activation parameters of the oxygenation reaction based on an observed reaction constant k_{obs} should be considered with caution. The overall fifth order rate dependence of the reaction on the reactants shows a complex molecularity of the catalytic cycle and the k_{obs} values determined do contain the preequilibrium constants K_1 and K_2 together with the k_3 value, assumed as the rate-determining step. Since the absolute value of these constants could not be determined the activation parameters should be handled with care. The value of the enthalpy of activation and the free enthalpy of activation are responsible for the relatively slow reacton rate however they are smaller than those obtained using the similar tmeda complex. These differ-



Scheme 1.

ences are in agreement with the kinetic data (greater initial velocity under similar conditions) obtained. According to the transition state theory [31] the formation of the activated complex (as a result of the intramolecular electron transfer from the flavonolate ligand to the copper ion, and possibly the coordination of more flavonolate ligands to copper) is more favorable in the presence of the soft bpy ligand than in the case of tmeda. The relatively great positive entropy of activation suggests that the activated complex does not have too much steric constrains and, since the entropy of the activated complex is greater than that of the reactants, the complex must be very loosely bound [31].

4. Summary

In contrary to the oxygenation of $[Cu^{I}(PPh_3)_2(fla)]$ [21], $[Cu^{II}(fla)_2]$ [16], and $[Cu^{II}(tmeda)(fla)]ClO_4$ [27] where the stoichiometric oxygenation showed simple overal second order rate expressions, and by their use as catalyst for the oxygenation of flavonol overall third order reaction rate equations were found the copper(II) flavonolate complex with bpy $[Cu^{II}(bpy)(fla)]ClO_4$ shows significant differences. The complexity of the reaction kinetics with the overall fifth order reaction rate expression suggests a different reaction rate with different molecularity. The reason for the difference as we believe is the soft bpy ligand. Bpy lends the driving force to the formation of $[Cu^{I}(bpy)(fla^{\cdot})]^{+}$ from $[Cu^{II}(bpy)(fla)]^{+}$ and the formation of $[Cu^{I}(fla)_3]^{2-}$ with the counter ion of $[Cu(bpy)_2]^{+}$ species as have been shown earlier [30], and this octahedral copper(II) flavonolate species serves as a key intermediate in the present system. The whole catalytic cycle may be drawn as shown in Scheme 1. Scheme 1 is based on the kinetic data and suggests that due to intramolecular electron transfer in $[Cu^{II}(bpy)(fla)]^{+}$ according to Eq. (3) $[Cu^{I}(bpy)(fla^{\cdot})]^{+}$ is formed in a fast preequilibrium, and two of them react with flaH reversibly to form the key compound $[Cu^{I}(fla^{\cdot})_2(fla)]$ (Eq. (4)). $[Cu^{I}(fla^{\cdot})_2(fla)]$ reacts then in the slowest step with two dioxygen molecules to yield the oxygenated primary product $[Cu^{I}(flaO_2^{\cdot})_2(fla)]$. Due to a reverse directed intramolecular electron shift $[Cu^{II}(flaO_2^{-})_2(fla)]^{-}$ species are formed, which via an intramolecular nucleophilic attack on 4-C=O yield the endoperoxide first and then the decomposed products with *O*-benzoylsalycilate ligand and carbon monoxide. Ligand substitution by flaH ends up then the catalytic cycle.

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