

# Kinetics and mechanism of the stoichiometric oxygenation of the ionic zinc(II) flavonolate complex [Zn(flavonolate)(idpa)]ClO<sub>4</sub> (flavonolate; idpa = 3,3'-iminobis(*N,N*-dimethylpropylamine))

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## Abstract

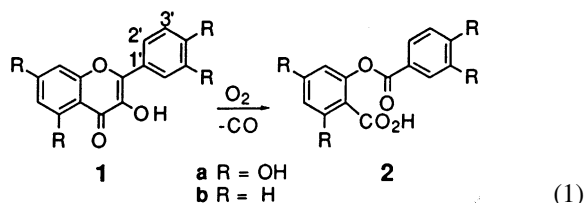
The preparation and characterization of [Zn<sup>II</sup>(4'-R-fla)(idpa)]ClO<sub>4</sub> (fla = flavonolate; idpa = 3,3'-iminobis(*N,N*-dimethylpropylamine)) complexes are described. Oxygenation of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> in dimethylformamide solution at elevated conditions gives [Zn<sup>II</sup>(idpa)(*O*-bs)]ClO<sub>4</sub> (*O*-bs = *O*-benzoylsalicylate) and carbon monoxide. The oxygenolysis of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> in DMF was followed by electronic spectroscopy and the rate constants were determined according to the rate law  $-d[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\text{ClO}_4]/dt = k_{\text{obs}} [\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\text{ClO}_4][\text{O}_2]$ . The rate constant, activation enthalpy and entropy at 373 K are as follows:  $k_{\text{obs}} (\text{M}^{-1} \text{s}^{-1}) = (3.11 \pm 0.12) \times 10^{-3}$ ,  $\Delta H^\ddagger (\text{kJ mol}^{-1}) = 75 \pm 5$ ,  $\Delta S^\ddagger (\text{J mol}^{-1} \text{K}^{-1}) = -96 \pm 13$ . The reaction fits a Hammett linear free energy relationship for 4'-substituted flavonols, and electron-releasing groups make the oxygenation reaction faster. In the course of the reaction O<sub>2</sub><sup>•-</sup> was formed suggesting a SET mechanism. [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> does not catalyze the oxygenation of flavonols. © 2001 Elsevier Science B.V. All rights reserved.

**Keywords:** Flavonol 2,4-dioxygenase; Quercetinase; Flavonol; Zinc complexes; Oxygenation; Kinetics; Dioxygenase

## 1. Introduction

Dioxygenation of various substrates with molecular oxygen are selectively catalyzed by different types of metalloenzymes. Oxygenases are metal-containing proteins and a fair number of them utilizes copper at their active sites [1–5]. 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. Fungi such as *Aspergillus* or *Pullularia* species oxidatively degrade quercetin (**1a**) and related flavonols into a depside and carbon monoxide (Eq. (1)) [6–9]. The copper-containing metalloenzyme flavonol 2,4-dioxygenase is responsible for this reaction [10].

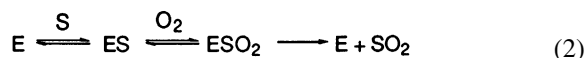


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In previous studies the intermediate formation of binary (ES) and ternary species (ESO<sub>2</sub>) has been proposed at the oxygenation by flavonol 2,4-dioxygenase as shown in Eq. (2) [11–13]. Many reports have appeared for the formation and structures of ES from both enzyme and model studies, but until now there has been only few mechanistic evidence for the formation of ESO<sub>2</sub> species. There are two types of



discussions on the structure of ES. One concerns whether flavonol coordinates to the metallic center as a bidentate ligand [11] or dissociates from the copper ion forming free flavonolate ion in the activation step [5]. The other concerns the change of the character of the flavonolate ligand, i.e. how the flavonolate ligand is activated for oxygenation: whether the flavonolate ligand can be regarded as a radical as shown in (A) or has an anionic character as in (B) (Fig. 1).

Extensive studies report on the coordination chemistry of flavonols of various metal ions. Recent crystallographic studies of flavonolato complexes of copper(I) [14], copper(II) [15–17], cobalt(III) [18], and zinc(II) [19,20] disclosed the coordination mode of the flavonolate ligand, geometries around the metal ions, and their influence on the delocalization of  $\pi$ -electrons in the flavonolate ligand. These complexes are important structural and functional models in studies mimicking dioxygenases.

Apart from the coordination mode of flavonolate ligands, it is important to know how the flavonolate ligand is activated for the reaction with molecular

oxygen. Model oxygenation reactions on quercetin and the parent compound flavonol have been carried out in order to understand the enzymic reaction. Base-catalyzed oxygenation of quercetin and related flavonols under aqueous [21], and nonaqueous conditions [22,23], and reactions with superoxide [24] have been investigated. Metal complexes of cobalt [25–27] and copper [28–30] have been found to act as catalysts for the oxygenation reaction. Copper(I) [31] and copper(II) [32–35] flavonolate complexes were also successfully used for the oxygenation of flavonol.

We have previously reported that [Cu<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> as a model for the ES complex is a good mimic for the flavonol 2,4-dioxygenase enzyme [17,35]. On the bases of kinetic measurements of the stoichiometric and catalytic oxygenation reaction we assumed that the redox active copper ion in the enzyme, and also in the model reactions, acts as an electron buffer, due to their ability to accept or donate electrons. In this sense the flavonolato ligand is transformed to a radical, and the copper(I) formed can activate dioxygen. In this paper we report details for the synthesis and characterization of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub>, which is a model for the ES complex with a nonredox metal zinc, and spectrophotometric studies aimed at elucidating the kinetics and mechanism of the stoichiometric oxygenation of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub>, to be compared with other model systems with redox metal ions in order to clear the possible role of the redox behavior of the metal ion.

## 2. Experimental

### 2.1. Instrumentation

IR spectra were recorded in either Nujol or KBr pellets on a Specord IR-75 (Carl Zeiss) spectrometer. Electronic spectra were measured on a Shimadzu UV-160 spectrometer using quartz cells. NMR spectra were recorded on a Varian Unity-300 spectrometer. <sup>1</sup>H NMR shifts are reported as values downfield from an external standard of Me<sub>4</sub>Si. GC analyses were performed on HP 5830A and HP 5890 gas chromatographs equipped with a flame ionization detector (CP SIL 8CB column), and a thermal conductivity detector (molecular sieve 5A column). GC–MS measurements were recorded on a HP 5890II/5971 GC/MSD at 75 eV.

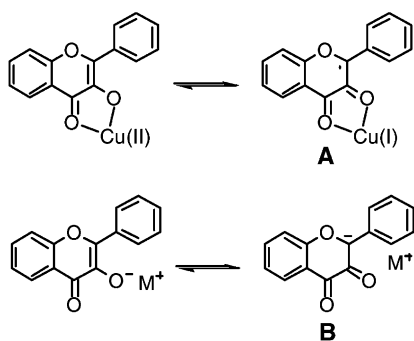


Fig. 1. Probable modes of coordination of flavonol in a flavonol-metal binary intermediate.

## 2.2. Materials and methods

Solvents [diethyl ether and DMF (HPLC grade with water content less than 0.03%)] were purchased from Aldrich in analytically pure quality. Diethyl ether was distilled from K–Na amalgam under Ar atmosphere. Flavonol [21], 4'-methoxyflavonol [21], 4'-methylflavonol [36], and 4'-chloroflavonol [36] were prepared by literature methods. The compounds 3,3'-iminobis(*N,N*-dimethylpropylamine) and  $\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  were obtained from Aldrich and used without further purification. Diazomethane [37] was freshly prepared according to the literature in ether and immediately used for the methylation reactions. Gaseous oxygen from Messergriesheim was 99.6% and passed through  $\text{P}_2\text{O}_5$  and Blaugel in order to remove traces of water and other impurities.

### 2.3. $[\text{Zn}^{\text{II}}(4'R\text{-fla})(\text{idpa})]\text{ClO}_4$ (**3**) (general procedure)

$\text{Zn}(\text{ClO}_4)_2 \cdot 6\text{H}_2\text{O}$  (3.724 g, 10 mmol) and 3,3'-iminobis(*N,N*-dimethylpropylamine) (2.3 cm<sup>3</sup>, 10 mmol) were dissolved in 150 cm<sup>3</sup> toluene–ethanol (1:1) and stirred under an atmosphere of argon for 1 h, then flavonol (2.38 g, 10 mmol) was added and the solution refluxed for 3 h. The solution was allowed to cool to room temperature and a yellow solid which deposited was then filtered off and recrystallized from toluene–ethanol (1:1) to give yellow crystals of (**3a**) (1.75 g, 30%): mp 231–233°C; UV–VIS ( $\lambda_{\text{max}}$ , DMF) 260 nm (log  $\epsilon$  4.17), 419 (4.32); IR (KBr) 3250, 1565, 1526, 1486, 1442, 1417, 1362, 1318, 1259, 1213, 1186, 1156, 1115, 1081, 1042, 999, 915, 757, 715, 692, 669, 629, 551, 497 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>CO, 22°C)  $\delta$  7.3–8.8 (m, 9H, ArH), 4.4 (s, 1H), 3.2–3.4 (m, 4H), 2.85–3.1 (m, 4H), 2.65 (s, 12), 2.05–2.15 (m, 4H). Anal. calcd. for C<sub>25</sub>H<sub>34</sub>N<sub>3</sub>O<sub>7</sub>ZnCl: C, 50.95; H, 5.81; N, 7.13. Found C, 50.43; H, 6.09; N, 6.94. Complexes  $[\text{Zn}^{\text{II}}(4'R\text{-fla})(\text{idpa})]\text{ClO}_4$  (**3b–d**) were prepared by the same method as that described above for the preparation of (**3a**).

### 2.4. $[\text{Zn}^{\text{II}}(4'Me\text{-fla})(\text{idpa})]\text{ClO}_4$ (**3b**)

Yield: 4.53 g, 75%; mp 197–199°C; UV–VIS ( $\lambda_{\text{max}}$ , DMF) 261.5 nm (log  $\epsilon$  4.106), 422 (4.208); IR

(nujol) 3235, 1564, 1520, 1341, 1273, 1253, 1240, 1197, 1104, 1065, 982, 946, 919, 863, 833, 766, 690, 633, 553, 499 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>CO, 22°C)  $\delta$  7.3–8.8 (m, 8H, ArH), 4.4 (s, 1H), 3.2–3.4 (m, 4H), 2.85–3.1 (m, 4H), 2.65 (s, 12H), 2.4 (s, 3H), 2.05–2.15 (m, 4H). Anal. calcd. for C<sub>26</sub>H<sub>36</sub>N<sub>3</sub>O<sub>8</sub>ZnCl: C, 51.75; H, 6.01; N, 6.96. Found C, 50.94; H, 5.93; N, 6.88.

### 2.5. $[\text{Zn}^{\text{II}}(4'Cl\text{-fla})(\text{idpa})]\text{ClO}_4$ (**3c**)

Yield: 4.35 g, 70%; mp 241–243°C; UV–VIS ( $\lambda_{\text{max}}$ , DMF) 262.5 nm (log  $\epsilon$  4.18), 419.5 (4.30); IR (KBr) 3247, 1553, 1537, 1473, 1411, 1351, 1318, 1233, 1214, 1177, 1144, 1102, 1063, 1041, 1002, 964, 908, 843, 752, 681, 638, 548, 495 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>CO, 22°C)  $\delta$  7.3–8.8 (m, 8H, ArH), 4.4 (s, 1H), 3.2–3.4 (m, 4H), 2.85–3.1 (m, 4H), 2.65 (s, 12H), 2.05–2.15 (m, 4H). Anal. calcd. for C<sub>25</sub>H<sub>33</sub>N<sub>3</sub>O<sub>7</sub>ZnCl<sub>2</sub>: C, 48.13; H, 5.33; N, 6.74. Found C, 47.52; H, 5.18; N, 6.38.

### 2.6. $[\text{Zn}^{\text{II}}(4'OMe\text{-fla})(\text{idpa})]\text{ClO}_4$ (**3d**)

Yield: 4.95 g, 80%; mp 225–227°C; UV–VIS ( $\lambda_{\text{max}}$ , DMF) 266 nm (log  $\epsilon$  4.212), 427 (4.32); IR (KBr) 3247, 1556, 1502, 1492, 1416, 1363, 1316, 1256, 1217, 1176, 1156, 1116, 1043, 920, 836, 762, 683, 629, 576 cm<sup>-1</sup>; <sup>1</sup>H NMR ((CD<sub>3</sub>)<sub>2</sub>CO, 22°C)  $\delta$  7.3–8.8 (m, 8H, ArH), 4.4 (s, 1H), 3.84 (s, 3H), 3.2–3.4 (m, 4H), 2.85–3.1 (m, 4H), 2.65 (s, 12H), 2.05–2.15 (m, 4H). Anal. calcd. for C<sub>26</sub>H<sub>36</sub>N<sub>3</sub>O<sub>8</sub>ZnCl: C, 50.41; H, 5.86; N, 6.78. Found C, 49.75; H, 5.76; N, 6.55.

### 2.7. Oxygenation of $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$ (**3a**)

0.295 g (0.5 mmol)  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  was dissolved in 15 cm<sup>3</sup> DMF and stirred at 100°C under dioxygen atmosphere for 10 h. The formation of  $[\text{Zn}^{\text{II}}(\text{idpa})(O\text{-bs})]\text{ClO}_4$  (**4a**) from  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  (**3a**) requires dioxygen but no apparent oxygen uptake was observed, because the absorption of dioxygen and the liberation of carbon monoxide compensate each other. The GC analysis of the gas phase showed 0.14 mmol CO (28% conversion). Approximately, 0.14 mmol of dioxygen has been consumed and as much carbon monoxide has been evolved during the reaction. 0.5 cm<sup>3</sup> water and 2 cm<sup>3</sup> diazomethane

solution (in diethyl ether) was added to 1 cm<sup>3</sup> of the reaction mixture at room temperature and the conversion of the coordinated flavonolate (35%) and the yields of the products *N,N*-dimethylbenzamide (14%), benzoic acid (20%), salicylic acid (34%) and *O*-benzoylsalicylic acid (32%) were determined by GC.

### 2.8. Kinetic measurements

Reactions of [Zn<sup>II</sup>(4'R-fla)(idpa)]ClO<sub>4</sub> complexes with O<sub>2</sub> were performed in DMF solutions. In a typical experiment [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> (**3**) was dissolved under argon atmosphere in a thermostatted reaction vessel with an inlet for taking samples with a syringe, and connected to mercury manometer to regulate constant pressure. The solution was then heated to the appropriate temperature. A sample was then taken by syringe, and the initial concentration of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> (**3**) was determined by UV–VIS spectroscopy measuring the absorbance of the reaction mixture at 419 nm (log ε 4.32) {λ<sub>max</sub> of a typical band of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub>}. The argon was then replaced by dioxygen and the consumption of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> was analyzed periodically

(ca. every 10 min). Experimental conditions are summarized in Table 1. The temperature was determined with an accuracy of ±0.5°C and the pressure of dioxygen with an accuracy of ±0.5%. The O<sub>2</sub> concentration was calculated from literature data [38] taking into account the partial pressure of DMF [39] and assuming the validity of Dalton's law.

### 3. Results and discussion

The kinetics of the oxygenation reaction in DMF was followed by determining the concentration of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> (**3a**) in the reaction solution as a function of time by UV–VIS spectroscopy under constant dioxygen pressure, determining the variation of the absorbance of the flavonolate band at 419 nm. The solvent had a very low water content (below 0.03%), however by rising the water concentration to 2.38 × 10<sup>-2</sup> M by addition of water did not change the reaction rate (Table 1; experiments 12). A simple rate law for the reaction between [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> (**3a**) and O<sub>2</sub> is given in Eq. (2). In order to determine the rate dependence on the various reactants, oxygenation runs were performed at various initial

Table 1  
Kinetic data for the oxygenation of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> in DMF solution

Experiment number	Temperature (°C)	10 <sup>3</sup> [O <sub>2</sub> ] (mol dm <sup>-3</sup> )	10 <sup>4</sup> [Zn] (mol dm <sup>-3</sup> ) <sup>a</sup>	10 <sup>5</sup> <i>k</i> (s <sup>-1</sup> )	<i>R</i> (%) <sup>b</sup>	10 <sup>3</sup> <i>k</i> <sub>obs</sub> (s <sup>-1</sup> mol <sup>-1</sup> dm <sup>3</sup> )	10 <sup>8</sup> d[Zn]/dt (mol dm <sup>-3</sup> s <sup>-1</sup> )
1	100	7.70	3.42	2.58	99.85	3.35 ± 0.05	0.88
2	100	7.70	4.83	2.10	99.79	2.73 ± 0.07	1.01
3	100	7.70	9.81	2.20	99.50	2.86 ± 0.08	2.16
4	100	7.70	14.13	2.54	99.53	3.30 ± 0.10	3.59
5	100	1.54	14.13	0.60	99.28	3.89 ± 0.18	0.85
6	100	4.50	14.25	1.50	99.48	3.33 ± 0.10	2.14
7	100	5.50	14.58	1.59	99.68	2.89 ± 0.08	2.32
						3.11 ± 0.12 <sup>c</sup>	
8	110	7.45	13.45	3.87	99.83	5.20 ± 0.12	
9	120	6.96	10.94	6.93	99.37	9.95 ± 0.45	
10	130	6.54	12.60	12.99	99.97	19.87 ± 0.32	
11	100	7.70	4.88	5.64	99.09	73.19 ± 5.12 <sup>d</sup>	
12	100	7.70	7.15	2.39	99.85	3.10 ± 0.05 <sup>e</sup>	

<sup>a</sup> In 50 cm<sup>3</sup> DMF.

<sup>b</sup> Correlation coefficients of least-squares regressions.

<sup>c</sup> Mean value of the kinetic constant *k* and its standard deviation σ(*k*) were calculated as  $k = (\sum_i w_i k_i / \sum_i w_i)$  and  $\sigma(k) = (\sum_i w_i (k_i - k)^2 / (n - 1) \sum_i w_i)^{1/2}$ , where  $w_i = 1/\sigma_i^2$ .

<sup>d</sup> 48 cm<sup>3</sup> DMF–2 cm<sup>3</sup> py.

<sup>e</sup> In the presence of added water [H<sub>2</sub>O] = 2.38 × 10<sup>-2</sup> M.

substrate concentrations (Table 1; experiments 1–4)

$$\begin{aligned} & \frac{d[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]}{dt} \\ &= \frac{d[\{\text{Zn}^{\text{II}}(\text{O-bs})(\text{idpa})\}\text{ClO}_4]}{dt} \\ &= k[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]^m [\text{O}_2]^n \end{aligned} \quad (2)$$

and at different dioxygen pressures (Table 1; experiments 4–7 under pseudo first order conditions with a constant dioxygen pressure). Eq. (2) can then have a more simple feature as written in Eq. (3),  $k'$  being the pseudo first order rate constant.

$$\begin{aligned} & \frac{d[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]}{dt} \\ &= k'[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]^m \end{aligned} \quad (3)$$

Measuring the time dependence of the change of concentration of  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  (**3a**) during the oxygenation shows that plots of  $\log[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]$  versus time were linear in experiments 1–4, indicating that the reaction is first order with respect to substrate concentration. Columns  $k'$  and  $R$  in Table 1 report slopes and the correlation coefficients obtained by the least-squares method for these linear regressions. A typical first order plot for experiment 3 shows that the reaction remains first order for the whole time in which the experiment was followed (58% conversion, 6.5 h).

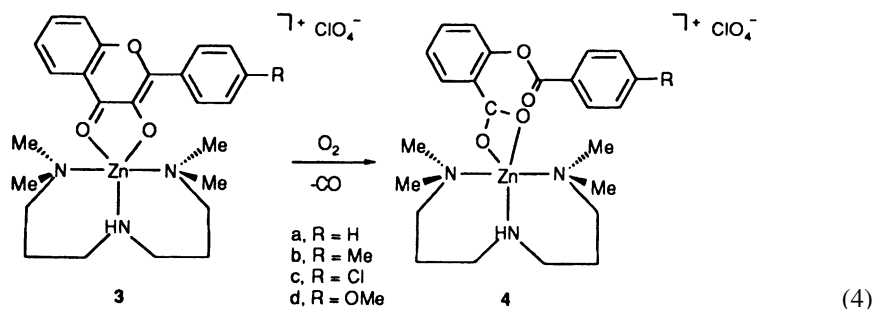
From variations of the reaction rates, plots of  $-d[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]/dt$  versus  $[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]$  were also linear in experiments 1–4 with a correlation coefficient of 99.38%, reinforcing that the reaction is indeed first order with respect to substrate concentration.

the validity of Dalton's law, the dissolved concentration of  $\text{O}_2$  being  $7.7 \times 10^{-3} \text{ mol dm}^{-3}$  at  $100^\circ\text{C}$  and 760 mm Hg  $\text{O}_2$  pressure) show that the dioxygen concentration affects the rate of the reaction (experiments 4–7; columns  $k'$  and  $R$  in Table 1), and that the reaction is first order with respect to dioxygen concentration.

On the basis of the results above, one can conclude that the oxygenation of  $[\{\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})\}\text{ClO}_4]$  obeys an overall second order rate equation with  $m = n = 1$  (Eq. (2)), from which a mean value of the kinetic constant  $k_{\text{obs}}$  of  $(3.11 \pm 0.12) \times 10^{-3} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$  at 373.16 K was obtained (Table 1). Addition of small amount of pyridine to the reaction mixture accelerated the reaction rate to  $k_{\text{obs}} = (7.32 \pm 0.51) \times 10^{-2} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$  with respect to  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  (experiment 11 in Table 1).

The activation parameters for the oxygenation reaction were determined from the temperature dependence of the kinetic constant  $k_{\text{obs}}$ . The temperature-dependent reaction rate measurements in the range of 373.16–403.16 K (experiments 1–7, 8–10 in Table 1) resulted in a straight line in the Eyring plot, with a correlation coefficient of 99.58% and activation parameters  $E_a = 78 \pm 5 \text{ kJ mol}^{-1}$ ,  $\Delta H^\ddagger = 75 \pm 5 \text{ kJ mol}^{-1}$  and  $\Delta S^\ddagger = -96 \pm 13 \text{ J mol}^{-1} \text{ K}^{-1}$ , respectively. Though activation parameters are often not discriminating factors in recognizing the reaction pathway, the negative entropy of activation ( $\Delta S^\ddagger$ ), however, clearly indicates an associative mode of activation in the rate-determining step.

Reaction rates on the oxygenation of 4'-substituted flavonolato zinc complexes ( $[\text{Zn}^{\text{II}}(4'\text{-R-fla})(\text{idpa})]\text{ClO}_4$  (**3**) Eq. (4)) under identical conditions were determined



Experiments made at different dioxygen concentrations (calculated from literature data assuming

(with various electron-withdrawing or releasing substituents R) in order to find out electronic effects

Table 2  
Hammett data for the oxygenation of  $[\text{Zn}^{\text{II}}(4\text{R}'\text{-fla})(\text{idpa})]\text{ClO}_4$

Complex	R	$\sigma$	$10^2 k$ ( $\text{s}^{-1} \text{mol}^{-1} \text{dm}^3$ ) <sup>a</sup>
<b>3a</b>	H	0.000	$1.00 \pm 0.05$
<b>3b</b>	$\text{CH}_3$	-0.170	$1.84 \pm 0.06$
<b>3c</b>	Cl	+0.227	$0.78 \pm 0.01$
<b>3d</b>	$\text{OCH}_3$	-0.268	$1.81 \pm 0.05$

<sup>a</sup> In DMF at 120°C.

on the reaction rate (Table 2). The Hammett plot obtained is shown in Fig. 2. Since the rate constants of all the substituents fall on a single line, we conclude that there is no change in the mechanism over the series of zinc flavonolates studied. The linearity in Fig. 2 demonstrates a linear free-energy relationship [40], and the reaction constant  $\rho$  was found to be -0.83. The observation that  $\rho$  is large and negative supports the contention that the substituents at the *para*-position have a significant electronic effect at the remote reaction center. Electron-donating substituents increase the reaction rate, while electron-withdrawing substituents slow down the reaction. These results suggest that the dioxygen species at the transition state should be electrophilic.

A reaction mechanism that fits the chemical, spectroscopic, kinetic and thermodynamic data is shown in Scheme 1. Flavonol and quercetin are assumed to be reactive substrates in the enzyme reaction [11–13]. However, it is also known that these substrates are also inert towards dioxygen in their protonated forms.

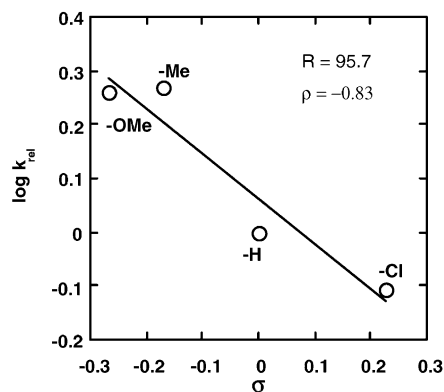
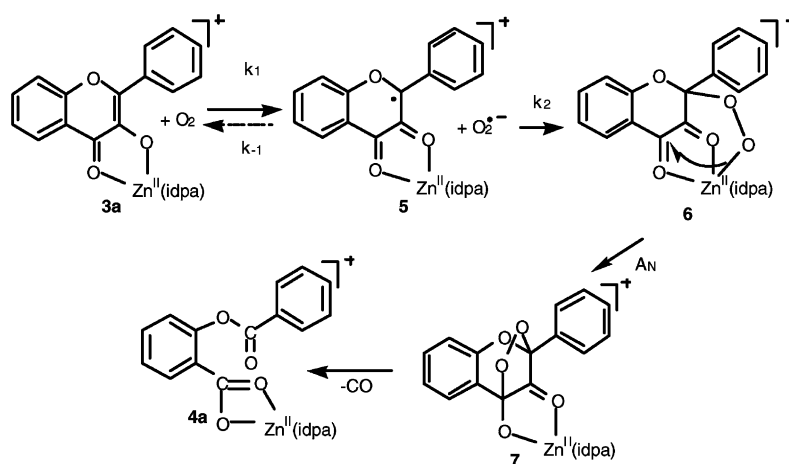


Fig. 2. Hammett plot for the oxygenation of  $[\text{Zn}^{\text{II}}(4\text{R}'\text{-fla})(\text{idpa})]\text{ClO}_4$  complexes (Table 2).

Deprotonation of flavonols enhances the reactivity of the corresponding anions toward dioxygen [27]. On the bases of the kinetic, CV and EPR studies a single electron transfer (SET) was assumed for the oxygenation of potassium flavonolate. The key step was thought to be the formation of a flavonoxo radical in a bimolecular process followed by the reaction of the flavonoxo radical with the superoxide ion [23]. Flavonolate as a chelating ligand forms a stable complex with zinc ion in the presence of 3,3'-iminobis(*N,N*-dimethylpropylamine). The Zn(II) center is in distorted trigonal-bipyramidal environment. Due to coordination of flavonolate to the zinc there are changes in the bond lengths of the pyranone



Scheme 1.

ring, which may be assigned to delocalization of the  $\pi$ -system over the whole molecule [20]. Kinetic studies on the oxygenation of the flavonolato zinc complexes established a second-order overall rate expression, indicating that the rate-determining step must be bimolecular, namely the reaction of  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  (**3a**) with dioxygen. Reduction of nitroblue tetrazolium (NBT) to blue formazan was used as an indicator for superoxid anion formation and this test indicated its presence. Addition of NBT to the reaction mixture, the yellow color turns to intense green and a new intense band at 521 nm arises. This absorption has been assigned to formazan signaling the presence of superoxide anion [41]. On that basis we believe that the oxygenolysis of flavonolate complexes with nonredox metals, such as  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$ , in aprotic solvents has a single electron transfer (SET) mechanism. The second order overall rate equation can be interpreted by two mechanistic pathways with different rate-determining steps, both satisfying the kinetic data (Scheme 1). In the first case the slow reaction of  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  with  $\text{O}_2$  is an irreversible rate-determining electron transfer from the substrate to  $\text{O}_2$  forming the flavonoxyl radical complex **5** and superoxide ion. This is followed by a fast radical radical coupling reaction of the flavonoxyl radical (**5**) and superoxide ion to give a deprotonated hydroperoxide species (**6**), which after an intramolecular An reaction on  $4\text{C}=\text{O}$  leads to an unstable endoperoxide (**7**), and its decomposition results then in the *O*-benzoylsalicylato zinc(II) complex and carbon monoxide. All attempts to isolate or detect the endoperoxide **7** failed. That suggest that this type of endoperoxides (**7**) are very unstable if compared to other endoperoxides [42] where the elimination of CO is not possible. In the second case it can be assumed that  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  undergoes electron transfer to  $\text{O}_2$  in a fast reversible step resulting in the flavonoxyl radical complex **5** and superoxide ion. This pre-equilibrium lies very much on the side of the starting reagents ( $k_1/k_{-1}$  being small). This is followed then by the rate-determining step where  $[\text{Zn}^{\text{II}}(\text{fla}^\bullet)(\text{idpa})]\text{ClO}_4$  reacts with  $\text{O}_2^{\bullet-}$ . All consecutive steps are fast and identical with those outlined before. Applying steady-state treatment for this mechanism,  $[\text{Zn}^{\text{II}}(\text{fla}^\bullet)(\text{idpa})]\text{ClO}_4/dt = 0$ , the rate Eq. (5) can be deduced. Rearranging Eq. (5) the rate equation can have the form as shown in Eq. (6). This

can be simplified then further to a single second order rate equation by assuming  $k_2/k_{-1}$  and  $K_1[\text{O}_2]/[\text{O}_2^{\bullet-}]$  being much smaller than 1.

$$\frac{d\{[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4\}}{dt} = \frac{k_1 k_2 \{[\text{Zn}^{\text{II}}(\text{fla}^\bullet)(\text{idpa})]\text{ClO}_4\} [\text{O}_2] [\text{O}_2^{\bullet-}]}{k_1 [\text{O}_2] + (k_{-1} + k_2) [\text{O}_2^{\bullet-}]} \quad (5)$$

$$\frac{d\{[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4\}}{dt} = \frac{K_1 k_2 \{[\text{Zn}^{\text{II}}(\text{fla}^\bullet)(\text{idpa})]\text{ClO}_4\}_0 [\text{O}_2]}{k_2/k_{-1} + 1 + K_1 [\text{O}_2]/[\text{O}_2^{\bullet-}]} = k_{\text{obs}} \{[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4\}_0 [\text{O}_2] \quad (6)$$

As a conclusion it can be said that the interaction between the bidentate flavonolate ligand and the Lewis acid zinc(II) ion leads to lowering the electron density on the flavonolate ligand, which causes a decrease in reaction rate of the single electron transfer from the Zn(II)-coordinated flavonolate to dioxygen or the radical radical coupling of **5** and  $\text{O}_2^{\bullet-}$ . This is supported by the Hammett correlation and a rate-accelerating effect of added pyridine on the reaction. In the oxygenation reaction of the flavonolate in various systems the reaction rates are in the following order:  $n\text{-Bu}_4\text{Nfla} > \text{flaK} > [\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4 + \text{py} > [\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4 < [\text{Cu}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  (Table 3). The small difference in  $\rho$  values observed for Zn and potassium-containing systems suggests that the mechanism of the two oxygenation reactions are the same. The higher electron density on the flavonolate increases the reducing power of the enolate or carbanion form (Fig. 1) making the electron transfer easier or shifting the pre-equilibrium more to the side of **5** and  $\text{O}_2^{\bullet-}$ , that means that  $K_1$  becomes bigger. The difference in reaction rates observed for  $[\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})]\text{ClO}_4$  in the presence and absence of py indicates that there are two pathways for the oxygenation, namely a slower process in DMF where the idpa ligand is bonded to zinc, and a faster one in the presence of an excess pyridine, where the ligand idpa is probably replaced by the strongly ligating pyridine, while the flavonolate remains bonded to the zinc ion. That is supported by conductivity measurements too, where the conductivity of the solution ( $\Lambda_0 = 35 \text{ cm}^2 \text{ mol}^{-1} \text{ ohm}^{-1}$ ) does not change, excluding the formation of new ions.

Table 3

Oxygenation rates and activation parameters for fla(*n*-Bu<sub>4</sub>N), flaK, [Zn<sup>II</sup>(4'R-fla)(idpa)]ClO<sub>4</sub> and [Cu<sup>II</sup>(4'R-fla)(idpa)]ClO<sub>4</sub>

Complex	$k_{\text{obs}}$ (s <sup>-1</sup> mol <sup>-1</sup> dm <sup>3</sup> )	$\Delta H^{\ddagger}$ (kJ mol <sup>-1</sup> )	$\Delta S^{\ddagger}$ (J mol <sup>-1</sup> K <sup>-1</sup> )	$\rho$
fla( <i>n</i> -Bu <sub>4</sub> N) <sup>a</sup>	$(5.18 \pm 0.16) \times 10^{-1}$			
flaK <sup>a</sup>	$(3.28 \pm 0.10) \times 10^{-1}$	29 ± 2	-161 ± 6	-0.73
[Zn <sup>II</sup> (fla)(idpa)]ClO <sub>4</sub> <sup>b</sup>	$(7.32 \pm 5.12) \times 10^{-2}$			
[Zn <sup>II</sup> (fla)(idpa)]ClO <sub>4</sub> <sup>c</sup>	$(3.11 \pm 0.12) \times 10^{-3}$	75 ± 5	-96 ± 13	-0.83
[Cu <sup>II</sup> (fla)(idpa)]ClO <sub>4</sub>	$(6.13 \pm 0.16) \times 10^{-3}$	64 ± 5	-120 ± 13	-0.29

<sup>a</sup> 40°C, DMF.<sup>b</sup> 100°C, DMF + py.<sup>c</sup> 100°C, DMF.

In comparison of the two mechanism we believe that the one with the rate-determining step of **5** and O<sub>2</sub><sup>•-</sup> is operative. Recent data on the rate constant of the electron transfer reaction from C<sub>60</sub>-anion radical (C<sub>60</sub><sup>•-</sup>) to molecular oxygen was found to be  $(3.7 \pm 0.2) \times 10^2 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$  [43]. The relatively slow rate was explained by the endothermic process ( $\Delta G_{\text{et}} > 0$ ) based on CV measurements. In our case the rate constant is three order of magnitude smaller  $(3.11 \pm 0.12) \times 10^{-3} \text{ M}^{-1} \text{ s}^{-1}$ . Despite the fact that the  $\Delta G$  value is even bigger in this case (based on calculation of the CV values [23]) and so an even slower reaction rate could be expected, we count on a mechanism in which the complex **3a** reacts with O<sub>2</sub> in a fast pre-equilibrium and the reaction of [Zn<sup>II</sup>(fla<sup>•</sup>)(idpa)]ClO<sub>4</sub> with O<sub>2</sub><sup>•-</sup> is rate-determining.

Attempts to use [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> as a catalyst for the oxygenation of flavonol failed. The reason for that can be either that the flavonol cannot be replaced by the product *O*-benzoylsalicylic acid and so the catalytic cycle is disrupted, or **3a** reacts with an excess of flavonol to an unreactive species, which is a pitfall in the cycle. Spectrophotometric monitoring of the reaction of **3a** with flavonol under argon proved the second case to be true. As shown in Fig. 3 the time profile of the reaction indicates the formation of a new species by increasing absorbance at 419 nm and decreasing intensities at 342.5 nm with isobestic points at 375.5 and 288 nm. The increase of the band at 419 nm, characteristic for the flavonolate ion, suggests that further 4 moles of flavonolate ions are formed at the end of the reaction. This may be interpreted as shown in Eq. (7).

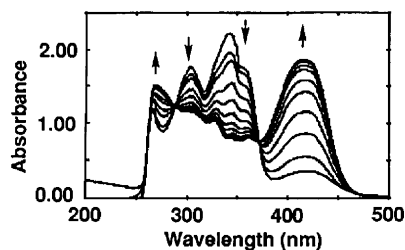
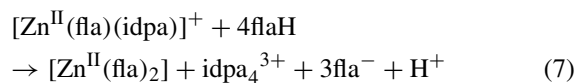


Fig. 3. Spectral changes at the reaction of [Zn<sup>II</sup>(fla)(idpa)]ClO<sub>4</sub> with excess flavonol. [Zn]<sub>0</sub> =  $1.5 \times 10^{-4}$  M, [flaH]<sub>0</sub> =  $6.0 \times 10^{-4}$  M,  $T = 130^\circ\text{C}$ , 50 cm<sup>3</sup> DMF, Ar.

**3a** is probably dismantled during the reaction, while Zn<sup>II</sup>(fla)<sub>2</sub> is formed and the basic ligand idpa deprotonates three further flavonol molecules. The species with the flavonolate ions so formed are much less reactive towards dioxygen than **3a**.

The above results indicate that the copper ion at the active site of quercetinase and in model systems undergoes valence tautomerism Cu<sup>II</sup>(fla<sup>-</sup>) ↔ Cu<sup>I</sup>(fla<sup>•</sup>) (in a similar manner as in the case of Cu<sup>II</sup>(catecholate) ↔ Cu<sup>I</sup>(semiquinone) systems [44]), and the Cu<sup>I</sup>(fla<sup>•</sup>) species reacts with dioxygen to the superoxo copper complex Cu<sup>II</sup>(O<sub>2</sub>)(fla<sup>•</sup>). In model studies with Zn(II) valence tautomerism is excluded, however the coordinated flavonolate ligand in Zn<sup>II</sup>(fla) undergoes electron transfer to O<sub>2</sub> to give Zn<sup>II</sup>(fla<sup>•</sup>) and free superoxide ion. The free O<sub>2</sub><sup>•-</sup> could be proved but the detection of Zn<sup>II</sup>(fla<sup>•</sup>) failed by EPR due to its instability. However, the reaction of Zn<sup>II</sup>(fla<sup>•</sup>) with O<sub>2</sub><sup>•-</sup> and consecutive reactions (see Scheme 1) lead to the endoperoxide and to the zinc complex of *O*-benzoylsalicylic acid Zn<sup>II</sup>(*O*-bs)(idpa)<sup>+</sup>. In that case a different mechanism is operative resulting in the same products. It is also interesting to note in the



case of the Zn model, contrary to its copper analogue, the exchange of the product ligand *O*-bs by fla is not possible in attempted catalytic reactions, which means that  $\text{Zn}^{\text{II}}(\text{fla})(\text{idpa})^+$  does not catalyze the oxygenation of flavonol. This is explained by the formation of new, stable, inactive zinc flavonolate complexes.

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### References

- [1] B.G. Fox, J.D. Lipscomb, in: C. Reddy, G.A. Hamilton (Eds.), *Biological Oxidation Systems*, Academic Press, New York, 1990, p. 367.
- [2] K.D. Karlin, Z. Tyeklár, *Bioinorganic Chemistry of Copper*, Chapman & Hall, New York, 1992.
- [3] E.I. Solomon, M.J. Baldwin, M.D. Lowery, *Chem. Rev.* 92 (1992) 521.
- [4] K.D. Karlin, *Science* 261 (1993) 701.
- [5] T. Funabiki, *Catalysis by Metal Complexes, Oxygenases and Model Systems*, Kluwer Academic Press, Dordrecht, The Netherlands, 1997.
- [6] D.W. Westlake, G. Talbot, E.R. Blakley, F.J. Simpson, *Can. J. Microbiol.* 5 (1959) 62.
- [7] F.J. Simpson, G. Talbot, D.W. Westlake, *Biochem. Biophys. Res. Commun.* 2 (1959) 621.
- [8] S. Hattori, I. Noguchi, *Nature* 184 (1959) 1145.
- [9] H.G. Krishnamurty, F.J. Simpson, *J. Biol. Chem.* 245 (1970) 1467.
- [10] T. Oka, F.J. Simpson, H.G. Krishnamurty, *Can. J. Microbiol.* 18 (1972) 493.
- [11] E. Makasheva, N.T. Golovkina, *Zh. Obsch. Khim.* 43 (1973) 1640.
- [12] M. Thomson, C.R. Williams, *Anal. Chim. Acta* 85 (1976) 375.
- [13] K. Takamura, M. Ito, *Chem. Pharm. Bull.* 25 (1977) 3218.
- [14] G. Speier, V. Fülöp, L. Párkányi, *J. Chem. Soc., Chem. Commun.* (1990) 512.
- [15] É. Balogh-Hergovich, G. Speier, G. Argay, *J. Chem. Soc., Chem. Commun.* (1991) 551.
- [16] I. Lippai, G. Speier, G. Huttner, L. Zsolnai, *Acta Crystallogr. C53* (1997) 1547.
- [17] É. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner, L. Zsolnai, *Inorg. Chim. Acta* 304 (2000) 72.
- [18] W. Hiller, A. Nishinaga, A.Z. Rieker, *Z. Naturforsch.* 47b (1992) 1185.
- [19] T.A. Annan, C. Peppe, D.G. Tuck, *Can. J. Chem.* 68 (1990) 423.
- [20] É. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner, P. Rutsch, *Acta Crystallogr. C55* (1999) 557.
- [21] A. Nishinaga, T. Tojo, H. Tomita, T.J. Matsuura, *J. Chem. Soc., Perkin Trans. 1* (1979) 2511.
- [22] V. Rajananda, S.B. Brown, *Tetrahedron Lett.* 22 (1981) 4331.
- [23] G. Speier, J. Kaizer, L. Barhács, *J. Org. Chem.* 65 (2000) 3449.
- [24] M.M.A. El-Sukkary, G. Speier, *J. Chem. Soc., Chem. Commun.* (1981) 745.
- [25] A. Nishinaga, T. Tojo, T. Matsuura, *J. Chem. Soc., Chem. Commun.* (1974) 896.
- [26] A. Nishinaga, N. Numada, K. Maruyama, *Tetrahedron Lett.* 30 (1989) 2257.
- [27] A. Nishinaga, T. Kuwashige, T. Tsutsui, T. Mashino, K. Maruyama, *J. Chem. Soc., Dalton Trans.* (1994) 805.
- [28] M. Utaka, M. Hojo, Y. Fujii, A. Takeda, *Chem. Lett.* (1984) 635.
- [29] M. Utaka, A. Takeda, *J. Chem. Soc., Chem. Commun.* (1985) 1824.
- [30] É. Balogh-Hergovich, G. Speier, *J. Mol. Catal.* 71 (1992).
- [31] É. Balogh-Hergovich, J. Kaizer, G. Speier, V. Fülöp, L. Párkányi, *Inorg. Chem.* 38 (1999) 3787.
- [32] É. Balogh-Hergovich, J. Kaizer, G. Speier, *Inorg. Chim. Acta* 256 (1997) 9.
- [33] É. Balogh-Hergovich, J. Kaizer, G. Speier, G. Argay, L. Párkányi, *J. Chem. Soc., Dalton Trans.* (1999) 3847.
- [34] É. Balogh-Hergovich, J. Kaizer, G. Speier, *J. Mol. Catal.* 159 (2000) 215.
- [35] G. Speier, J. Kaizer, J. Pap, L. Barhács, *Inorg. Chim. Acta*, in press.
- [36] M.A. Smith, R.M. Newman, R.A. Webb, *J. Heterocycl. Chem.* 5 (1966) 425.
- [37] F. Arndt, in: A.H. Blatt (Ed.), *Organic Syntheses*, Vol. 2, Wiley, New York, 1943, p. 165.
- [38] A. Kruis, *Landolt-Börnstein*, Board 4, Teil 4., Springer, Berlin, 1976, p. 269.
- [39] G. Ram, A.R. Sharaf, *J. Ind. Chem. Soc.* 45 (1968) 13.
- [40] J. March, *Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*, 4th Edition, Wiley, New York, 1992, pp. 278–286.
- [41] B.H.J. Bielski, H.W. Richter, *J. Am. Chem. Soc.* 110 (1988) 8085.
- [42] E.L. Clennan, C.S. Foote, in: W. Ando (Ed.), *Organic Peroxides*, Wiley, Chichester, 1992, p. 225.
- [43] T. Konishi, M. Fujitsuka, O. Ito, *Chem. Lett.* (2000) 202.
- [44] C.G. Pierpont, C.W. Lange, in: K.D. Karlin (Ed.), *Progress in Inorganic Chemistry*, Vol. 41, Wiley, New York, 1994, pp. 422–424.