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Kinetics and mechanism of the stoichiometric oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ [fla = flavonolate, idpa = 3,3′-imino-bis(*N*,*N*-dimethylpropylamine)] and the [Cu^{II}(fla)(idpa)]ClO$_4$-catalysed oxygenation of flavonol

László Barhács [a], József Kaizer [a,b], József Pap [a], Gábor Speier [a,b,*]

[a] *Department of Organic Chemistry, University of Veszprém, 8201 Veszprem, Hungary*
[b] *Research Group for Petrochemistry of the Hungarian Academy of Sciences, 8201 Veszprem, Hungary*

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Abstract

Oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ (fla = flavonolate; idpa = 3,3′-iminobis(*N*,*N*-dimethylpropylamine)) in dimethylformamide gives [Cu^{II}(idpa)(*O*-bs)]ClO$_4$ (*O*-bs = *O*-benzoylsalicylate) and CO. The oxygenolysis of [Cu^{II}(fla)(idpa)]ClO$_4$ in DMF was followed by electronic spectroscopy and the rate law $-\mathrm{d}[\{\mathrm{Cu^{II}(fla)(idpa)}\}\mathrm{ClO_4}]/\mathrm{d}t = k_{\mathrm{obs}}[\{\mathrm{Cu^{II}(fla)(idpa)}\}\mathrm{ClO_4}][\mathrm{O_2}]$ was obtained. The rate constant, activation enthalpy and entropy at 373 K are $k_{\mathrm{obs}} = 6.13 \pm 0.16 \times 10^{-3}\ \mathrm{M^{-1}\,s^{-1}}$, $\Delta H^{\ddagger} = 64 \pm 5\ \mathrm{kJ\,mol^{-1}}$, $\Delta S^{\ddagger} = -120 \pm 13\ \mathrm{J\,mol^{-1}\,K^{-1}}$, respectively. The reaction fits a Hammett linear free energy relationship and a higher electron density on copper gives faster oxygenation rates. The complex [Cu^{II}(fla)(idpa)]ClO$_4$ has also been found to be a selective catalyst for the oxygenation of flavonol to the corresponding *O*-benzoylsalicylic acid and CO. The kinetics of the oxygenolysis in DMF was followed by electronic spectroscopy and the following rate law was obtained: $-\mathrm{d}[\mathrm{flaH}]/\mathrm{d}t = k_{\mathrm{obs}}[\{\mathrm{Cu^{II}(fla)(idpa)}\}\mathrm{ClO_4}][\mathrm{O_2}]$. The rate constant, activation enthalpy and entropy at 403 K are $k_{\mathrm{obs}} = 4.22 \pm 0.15 \times 10^{-2}\ \mathrm{M^{-1}\,s^{-1}}$, $\Delta H^{\ddagger} = 71 \pm 6\ \mathrm{kJ\,mol^{-1}}$, $\Delta S^{\ddagger} = -97 \pm 15\ \mathrm{J\,mol^{-1}\,K^{-1}}$, respectively. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Copper complexes; Flavonolate complexes; Kinetics and mechanism; Dioxygenation

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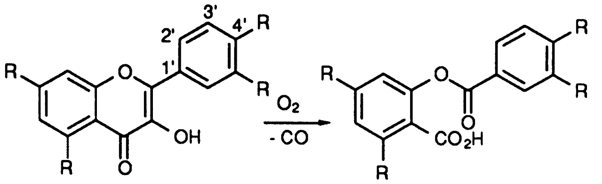
1. Introduction

Ring cleavage is the crucial step in the microbial degradation of aromatic and heterocyclic compounds. The intradiol and extradiol scission of catechols, catalysed by catechol dioxygenases, are well-characterised and studied nonheme iron- and copper-containing enzymes [1]. In contrast, most enzymes that catalyse the cleavage of heterocyclic compounds are less understood.

Our attention was directed to the biological oxygenation of quercetin (**1a**), which is oxidatively decarbonylated by the action of quercetinase. It has been reported that rutin is aerobically degraded to carbon monoxide and water-soluble products by the extracellular enzymes from *Aspergillus* and *Pullularia* species [2]. Rutin is first hydrolysed to quercetin (**1a**) and rutinose, then **1a** is oxidatively decarbonylated by the action of quercetinase to give a depside **2a** and carbon monoxide (Eq. (1)). The enzyme also catalyses the dioxygenation of the related flavonols.



1 **2**

a; R = OH
b; R = H

(1)

The enzymatic conversion of **1a** into **2a** was also examined by $^{18}\mathrm{O_2}$ labelling studies, and it was shown

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* Corresponding author. Tel.: +36-88-42-2022; fax: +36-88-42-7492.

E-mail address: speier@almos.vein.hu (G. Speier).

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that C-3 is liberated as carbon monoxide, while an oxygen molecule is incorporated at C-2 and C-4, but not into carbon monoxide, indicating that this unusual enzyme is indeed a 2,4-dioxygenase [3]. The catalytic centre of this dioxygenase contains copper(II) ion, but no information about the coordination environment around the active site is available and the role of the central metal ion has not yet been clarified. Quercetin (**1a**) was assumed to coordinate to Cu(II) as a chelating ligand at the 3-hydroxy and 4-carbonyl groups [4]. Studies on structural and functional models are important in order to elucidate the mechanism of the enzyme reaction. Base-catalysed oxygenation of quercetin and related flavonols under aqueous [5], and nonaqueous conditions [6,7], photosensitised oxygenations [8], and reactions with superoxide [9], have been investigated. Metal complexes of cobalt [10–12] and copper [13–15] have been found to act as catalysts for the oxygenation reaction.

We have been concerned with the synthesis of copper flavonolates and their quercetinase mimicking oxygenations [16–25]. We have previously reported the synthesis and characterisation of the copper(II) flavonolate (fla) complex with the tridentate ligand 3,3′-iminobis(*N*,*N*-dimethylpropylamine) (idpa), which at elevated temperature reacted with dioxygen to lead to the copper(II) complex of the corresponding depside (*O*-benzoylsalicylic acid, *O*-bsH) and CO. The structures of the complexes have been determined by X-ray diffraction. [Cu^{II}(fla)(idpa)]ClO$_4$ (**3**) exhibits a distorted trigonal-bipyramidal geometry ($\tau = 0.61$) around the copper(II) ion, while [Cu^{II}(idpa)(*O*-bs)]ClO$_4$ (**4**) has a distorted square-pyramidal arrangement ($\tau = 0.16$) [26]. In this paper we report volumetric and spectrophotometric studies aimed at elucidating the kinetics and mechanism of the stoichiometric oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3**) and the catalytic oxygenation of flavonol catalysed by [Cu^{II}(fla)(idpa)]ClO$_4$ (**3**).

2. Experimental

All manipulations were performed under pure dinitrogen or argon atmosphere unless otherwise stated using standard Schlenk-type inert gas technique [27]. Solvents (diethyl ether and DMF) were purchased from Aldrich in analytically pure quality and used as supplied. Flavonol [5], 4′-methylflavonol [28], 4′-methoxyflavonol [5], 4′-chloroflavonol [28] and [Cu^{II}(fla)(idpa)]ClO$_4$ [26] were prepared by literature methods. Diazomethane [29] was freshly prepared according to the literature in ether and used immediately for the methylation reactions. Gaseous dioxygen from Messergriesheim was 99.6% and passed through P_2O_5 and Blaugel in order to remove traces of water and other impurities.

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-160A spectrophotometer using quartz cells. GC analyses were performed on HP 5830A and HP 5890 gas chromatographs equipped with a flame ionisation detector (CP SIL 8CB column) and a thermal conductivity detector (molecular sieves 5A column). GC–MS measurements were recorded on a HP 5890II/5971 GC/MSD at 75 eV. Magnetic susceptibilities were determined on a Bruker-B-E 10B8 magnetometer. Reactions under controlled pressure of O_2 were performed with a double-wall reaction vessel equipped in a mercury manometer, inlet for sampling, and a Carl Zeiss (Jena) temperature controller.

2.2. Preparation of [Cu^{II}(4′R–fla)(idpa)]ClO$_4$ (**3**)

Cu^{II}(4′R–fla)(idpa)]ClO$_4$ (**3**) were prepared according to published procedures [26].

2.2.1. [Cu^{II}(4′Me–fla)(idpa)]ClO$_4$ (**3b**)

Yield: 0.51 g, 85%; m.p. 157–159 °C *Anal.* Found: C, 51.32; H, 5.91; N, 7.14. Calc. for $C_{26}H_{36}N_3O_7CuCl$: C, 51.91; H, 6.03; N, 6.98%. IR (KBr, cm^{-1}): 3225, 1561, 1519, 1489, 1413, 1359, 1298, 1258, 1237, 1189, 1160, 1119, 1078, 985, 941, 919, 859, 832, 764, 681, 636, 544, 499. UV–Vis: λ_{max} (nm) (log ε, mol^{-1} dm^3 cm^{-1}) (DMF): 262.5 (4.14), 429 (4.11), 731.5 (2.33). $\mu_B = 1.70$.

2.2.2. [Cu^{II}(4′Cl–fla)(idpa)]ClO$_4$ (**3c**)

Yield: 0.56 g, 90%; m.p. 163–65 °C. *Anal.* Found: C, 48.75; H, 4.94; N, 6.75. Calc. for $C_{25}H_{33}N_3O_7CuCl_2$: C, 48.28; H, 5.35; N, 6.76%. IR (nujol, cm^{-1}): 3234, 1561, 1555, 1321, 1265, 1245, 1225, 1188, 1165, 1108, 1081, 1041, 861, 820, 778, 761, 741, 697, 638, 587, 557, 528, 505. UV–Vis: λ_{max} (nm) (log ε, mol^{-1} dm^3 cm^{-1}) (DMF): 271.0 (4.24), 434.0 (4.27), 706.0 (2.37). $\mu_B = 1.97$.

2.2.3. [Cu^{II}(4′OMe–fla)(idpa)]ClO$_4$ (**3d**)

Yield: 0.49 g, 80%; m.p. 134–136 °C. *Anal.* Found: C, 50.04; H, 5.33; N, 7.23. Calc. for $C_{26}H_{36}N_3O_8CuCl$: C, 50.57; H, 5.88; N, 6.80%. IR (nujol, cm^{-1}): 3228, 1552, 1544, 1325, 1272, 1259, 1225, 1176, 1145, 1085, 1041, 1005, 976, 907, 845, 830, 817, 749, 717, 676, 619, 598, 544, 492. UV–Vis: λ_{max} (nm) (log ε, mol^{-1} dm^3 cm^{-1}) (DMF): 262.5 (4.14), 428.5 (3.92), 732.0 (2.56). $\mu_B = 1.73$.

2.3. Oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) in DMF

[Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) (0.294 g, 0.5 mmol) in DMF (50 cm^3) was treated with dioxygen (0.1 MPa) at

100 °C for 8 h, the solution was cooled to room temperature (r.t.) and diethyl ether was layered to afford a blue powder of [Cu^{II}(idpa)(*O*-bs)]ClO$_4$ (**4a**) (0.183 g, 62%) [26].

2.4. *Catalytic oxygenation of flavonol (**1b**) catalysed by [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**)*

Flavonol (**1b**) (0.119 g, 0.5 mmol) and [Cu^{II}(fla)-(idpa)]ClO$_4$ (**3a**) (0.083 g, 0.1 mmol) were dissolved and stirred at 100 °C in 20 cm^3 DMF for 8 h under dioxygen atmosphere. The formation of *O*-benzoylsalicylic acid (**2b**) from flavonol (**3a**) requires dioxygen but no apparent dioxygen uptake was observed since the absorption of oxygen and the liberation of carbon monoxide compensate each other. The GC analysis of the gas phase showed 0.24 mmol CO (47% conversion). Approximately 0.24 mmol of dioxygen was consumed and as much carbon monoxide evolved during the reaction. Diazomethane solution (2 cm^3) (in diethyl ether) was added to 1 cm^3 of the reaction mixture at r.t. and the conversion of flavonol (53%) and the yields of the products *N*,*N*-dimethylbenzamide (28%), benzoic acid (20%), salicylic acid (48%) and *O*-benzoylsalicylic acid (4%) were determined by GC as the methylated derivatives.

2.5. *Kinetic measurements*

Reactions of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) with O$_2$ were performed in DMF solutions. In a typical experiment [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) 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 O$_2$-pressure. The solution was then heated to the appropriate temperature. A sample was then taken by syringe, and the initial concentration of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) was determined by UV–Vis spectroscopy measuring the absorbance of the reaction mixture at 426.5 (log ε 4.27 [λ_{max} of a typical band of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**)] [26]. The argon was then replaced by dioxygen, and the consumption of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) was analysed periodically (approximately every 10 min). The experimental conditions are summarised in Table 1. The temperature and the pressure of dioxygen were determined with an accuracy of ± 0.5%. The O$_2$ concentration was calculated from literature data [30] taking into account the partial pressure of DMF [31] and assuming the validity of Dalton's law. The rate of oxygenation was independent of the stirring rate, excluding eventual diffusion control of the reaction.

Reactions of flavonol (**1b**) with O$_2$ catalysed by [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) were performed in DMF solutions by the same method as that described above for the stoichiometric oxygenation. The concentration of flavonol (**1b**) was determined by UV–Vis spectroscopy measuring the absorbance of the reaction mixture at 342.5 nm (log ε 4.24) (λ_{max} of a typical band of flavonol) [32,33].

3. Results

3.1. *Oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) in DMF*

The kinetics of the oxygenation reaction of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) was followed by determining its concentration in the reaction solution as a function of time by UV–Vis spectroscopy under constant dioxy-

Table 1
Kinetic data for the oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ in DMF solution

Exp. no.	Temp. (°C)	10^3[O$_2$] (mol dm^{-3})	10^4[Cu] [a] (mol dm^{-3})	$10^5k'$ (s^{-1})	R [b] (%)	10^3k_{obs} (s^{-1} mol^{-1} dm^3)	10^8d[Cu]/dt (mol dm^{-3} s^{-1})
1	100	7.70	3.99	4.65	99.28	6.03 ± 0.27	1.855
2	100	7.70	5.23	5.22	99.09	6.77 ± 0.27	2.729
3	100	7.70	8.44	5.43	98.90	7.05 ± 0.51	3.670
4	100	7.70	9.84	4.88	99.69	6.34 ± 0.17	5.339
5	100	7.70	14.00	4.35	99.53	5.65 ± 0.16	6.831
6	100	3.20	13.09	2.32	98.85	7.26 ± 0.53	3.041
7	100	10.00	12.86	6.00	99.48	6.00 ± 0.18	7.721
8	100	12.35	13.38	7.57	99.31	6.13 ± 0.28	10.129
						6.13 ± 0.16 [c]	
9	110	7.45	13.13	7.63	98.93	10.24 ± 0.71	
10	120	6.96	8.99	14.52	99.37	20.86 ± 0.94	
11	130	6.54	12.30	18.72	99.87	28.62 ± 0.46	

[a] In 50 cm^3 DMF.

[b] Correlation coefficients of least-squares regressions.

[c] Mean value of the kinetic constant k_{obs} and its standard deviation $\sigma(k)$ were calculated as $k_{obs} = (\Sigma_i w_i k_i/\Sigma_i w_i)$ and $\sigma(k) = (\Sigma_i w_i (k_i - k)^2/(n-1)\Sigma_i w_i)^{1/2}$, where $w_i = 1/\sigma_i^2$.

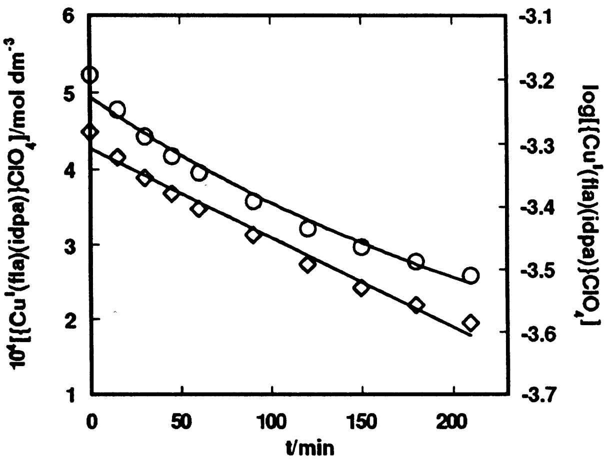


Fig. 1. Time course for the oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ in DMF (○) and plot of log[{Cu^{II}(fla)(idpa)}ClO$_4$] (◇) vs. reaction time for the oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ (experiment 2, Table 1).

Table 2
Hammett data for the oxygenation of [Cu^{II}(4′R–fla)(idpa)]ClO$_4$

Complex	R	σ	$10^2 k_{obs}$ [a] (s^{-1} mol^{-1} dm^3)
3a	H	0.000	2.09 ± 0.09
3b	CH_3	−0.170	2.37 ± 0.06
3c	Cl	+0.227	1.91 ± 0.06
3d	OCH_3	−0.268	2.67 ± 0.11

[a] In DMF at 120 °C.

gen pressure, determining the variation of the absorbance maxima of the flavonolate band at 426.5 nm. A simple rate law for the reaction between [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) and O$_2$ is given in Eq. (2):

$$-\mathrm{d}[\{Cu^{II}(fla)(idpa)\}ClO_4]/\mathrm{d}t = \mathrm{d}[\{Cu^{II}(O\text{-bs})(idpa)\}ClO_4]/\mathrm{d}t = k[\{Cu^{II}(fla)(idpa)\}ClO_4]^m[O_2]^n \quad (2)$$

In order to determine the rate dependence on the various reactants, oxygenation runs were performed at various initial substrate concentrations (Table 1; experiments 1–5) and at different dioxygen pressures (Table 1; experiments 5–8 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:

$$-\mathrm{d}[\{Cu^{II}(fla)(idpa)\}ClO_4]/\mathrm{d}t = k'[\{Cu^{II}(fla)(idpa)\}ClO_4]^m \quad (3)$$

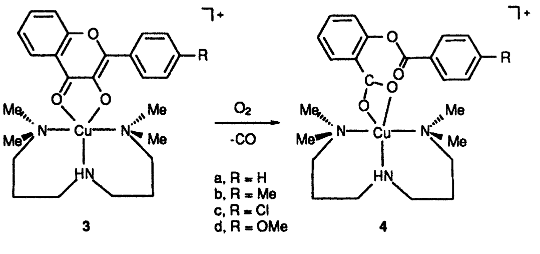
The time dependence of the change of concentration of [Cu^{II}(fla)(idpa)]ClO$_4$ (**3a**) during the oxygenation and linear plots of log[{Cu^{II}(fla)(idpa)}ClO$_4$] versus time in experiments 1–5 indicate that the reaction is first order with respect to substrate concentration. Columns of k' and R in Table 1 report slopes and the correlation coefficients obtained by the least-squares method for these linear regressions. A typical first-order plot is shown in Fig. 1 for experiment 2; the reaction remains first order for the whole time in which the experiment was followed (63% conversion, 2.5 h).

Variation of the reaction rates on the starting concentration of Cu^{II}(fla)(idpa)}ClO$_4$ shows that the plots of −d[{Cu^{II}(fla)(idpa)}ClO$_4$]/dt versus the initial [{Cu^{II}(fla)(idpa)}ClO$_4$] concentration were also linear in experiments 1–5 with a correlation coefficient of 99.13%, reinforcing that the reaction is indeed first order with respect to substrate concentration (Fig. S1).

Experiments conducted at different dioxygen concentrations (calculated from literature data assuming the validity of Dalton's law, the dissolved concentration of O$_2$ being 7.7×10^{-3} mol dm^{-3} at 100 °C and 760 mm Hg O$_2$ pressure) show that the dioxygen concentration affects the reaction rate (experiments 5–8; columns k' and R in Table 1) and that the reaction is also first order with respect to dioxygen concentration (Fig. S2). On the basis of the above results, one can conclude that the oxygenation of [Cu^{II}(fla)(idpa)]ClO$_4$ obeys an overall second-order rate equation with $m = n = 1$ (Eq. (2)), from which a mean value for the kinetic constant k_{obs} of $(6.13 \pm 0.16) \times 10^{-3}$ mol^{-1} dm^3 s^{-1} at 373.16 K was obtained (Table 1).

The activation parameters for the oxygenation reaction were determined from the temperature dependence of the kinetic constant k_{obs}. The temperature-dependent reaction rate measurements in the range of 363.16–393.16 K (experiments 1–11 in Table 1) resulted in a straight line in the Arrhenius plot (Fig. S3), with a correlation coefficient of 99.56% and activation parameters $E_a = 67 \pm 6$ kJ mol^{-1}, $\Delta H^{\ddagger} = 64 \pm 5$ kJ mol^{-1} and $\Delta S^{\ddagger} = -120 \pm 13$ J mol^{-1} K^{-1}.

Reaction rates on the oxygenation of 4′-substituted flavonolato copper complexes [Cu^{II}(4′R–fla)(idpa)]ClO$_4$ (**3**) (Eq. (4)) under identical conditions were determined (with various electron-withdrawing or releasing substituents R) in order to find out the electronic effects on the reaction rate (Table 2). The Hammett plot obtained is shown in Fig. 2. Electron-realising substituents enhanced the reaction rate, and the reaction constant ρ was found to be −0.29.



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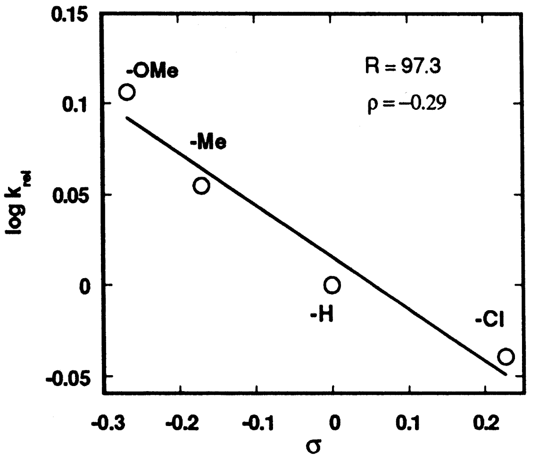


Fig. 2. Hammett plot for the oxygenation of $[Cu^{II}(4R'\text{-fla})(idpa)]ClO_4$ complexes (Table 2).

3.2. Catalytic oxygenation of flavonol (1b) catalysed by $[Cu^{II}(fla)(idpa)]ClO_4$ (3a)

The reaction between flavonol and dioxygen in the presence of catalytic amounts of $[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) were performed in DMF solution and examined in the temperature range from 397.16 to 412.16 K with a ratio between initial concentrations of $[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) and flavonol (**1b**) in the range 1:4–12. Experiments were also carried out at different dioxygen concentrations. Experimental conditions are summarised in Table 3.

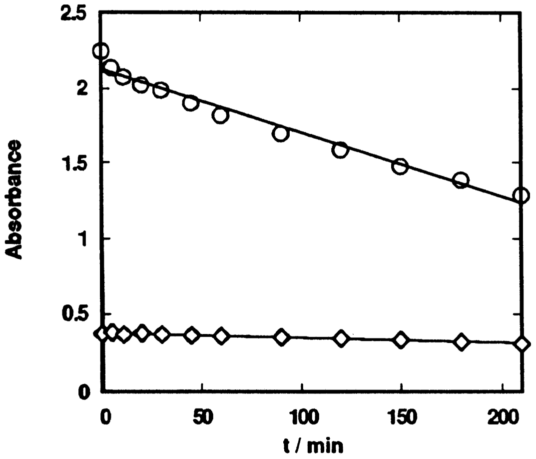


Fig. 3. Spectral changes accompanying the oxygenation of flaH at 342.5 (flaH) (○) and 426.5 nm $[Cu^{II}(fla)(idpa)]ClO_4$ (◇) during experiment 3 in Table 3.

$[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) exhibits absorption at 426.5 nm and flavonol shows an absorption band at 342.5 nm [32,33]. Spectral changes accompanying the reaction of flavonol with dioxygen in DMF solution show that the absorption peak at 342.5 nm decreases, while that at 426.5 nm does not change with time (Table 3; experiment 3) (Fig. 3).

A simple rate law for the catalytic reaction between flavonol (**1b**) and dioxygen catalysed by $[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) is given in Eq. (5):

Table 3
Kinetic data for the $[Cu^{II}(fla)(idpa)]ClO_4$ catalysed oxygenation of flavonol in DMF solution

Exp. no.	Temp. (°C)	$10^3[O_2]$ (mol dm^{-3})	10^4[Cu] [a] (mol dm^{-3})	10^4[flaH] [a] (mol dm^{-3})	$10^8k'$ (mol dm^{-3} s^{-1})	R [b] (%)	$10^{-2}k_{obs}$ (s^{-1} mol^{-1} dm^3)
1	130	6.54	0.525	6.19	1.120	99.18	3.26 ± 0.19
2	130	6.54	0.74	6.20	1.496	97.98	3.07 ± 0.76
3	130	6.54	0.93	6.22	2.162	99.47	3.54 ± 0.25
4	130	6.54	1.07	6.21	2.407	98.88	3.44 ± 0.23
5	130	6.54	1.36	6.10	3.383	99.52	3.80 ± 0.14
6	130	6.54	0.93	4.36	2.992	99.19	4.90 ± 0.31
7	130	6.54	0.93	4.87	2.580	99.82	4.22 ± 0.13
8	130	6.54	0.93	5.58	2.957	99.43	4.84 ± 0.21
9	130	6.54	0.93	7.03	2.523	99.44	4.13 ± 0.17
10	130	6.54	0.93	7.69	2.776	99.73	4.55 ± 0.12
11	130	3.27	0.93	5.96	1.071	99.16	3.28 ± 0.19
12	130	8.20	0.93	5.98	3.595	99.84	4.69 ± 0.11
13	130	9.43	0.93	6.00	4.219	99.69	4.80 ± 0.17
14	130	11.17	0.93	5.96	4.471	99.13	4.29 ± 0.32
							4.22 ± 0.15 [c]
15	124	7.02	0.93	6.01	2.158	99.74	3.29 ± 0.09
16	134	5.88	0.93	5.89	3.162	99.57	5.76 ± 0.20
17	139	4.35	0.93	5.99	2.982	99.24	7.34 ± 0.45

[a] In 50 cm^3 DMF.

[b] Correlation coefficients of least-squares regressions.

[c] Mean value of the kinetic constant k_{obs} and its standard derivation $\sigma(k)$ were calculated as $k = (\Sigma_i w_i k_i / \Sigma_i w_i)$ and $\sigma(k) = (\Sigma_i w_i (k_i - k)^2 / (n-1)\Sigma_i w_i)^{1/2}$, where $w_i = 1/\sigma_i^2$.

$$-\mathrm{d[flaH]/d}t = \mathrm{d}[O\text{-bsH}]/\mathrm{d}t = k[\mathrm{flaH}]^m[\{\mathrm{Cu^{II}(fla)(idpa)}\}\mathrm{ClO_4}]^n[\mathrm{O_2}]^q \quad (5)$$

In order to determine the rate dependence on the various reactants, oxygenation runs were performed at different substrate (Table 3; experiments 1–5), catalyst concentrations (Table 3; experiments 3, 6–10), and at different dioxygen pressures (Table 3; experiments 11–14). Assuming a constant concentration of the catalyst during each reaction, as well as a constant dioxygen pressure for the experiments the pseudo-first-order rate law (Eq. (6)) is obtained where $k' = k[\{\mathrm{Cu^{II}(fla)(idpa)}\}\mathrm{ClO_4}]^n[\mathrm{O_2}]^q$:

$$-\mathrm{d[flaH]/d}t = k'[\mathrm{flaH}]^m \quad (6)$$

Plots of [flaH] versus time were linear in experiments 1–14, indicating that the reaction is zero order with respect to substrate concentration. Columns k' and R in Table 3 report slopes and the correlation coefficients obtained by the least-squares method for these linear regressions. A typical zero-order plot is shown in Fig. 4 for experiment 3; the reaction remains zero order until 60% conversion (3.5 h).

Experiments with different initial flavonol (**1b**) concentrations show that flavonol (**1b**) does not appreciably affect the rate of the reaction (Table 3; experiments 3, 6–10). Plots of $-\mathrm{d[flaH]/d}t$ versus [flaH], as shown in Fig. S4, state also that the reaction is indeed zero order with respect to the substrate concentration. This means that $m = 0$ in Eq. (5).

Kinetic measurements of the reaction rate with respect to catalyst concentration (Table 3; experiments 1–5) indicate a first-order dependence. A plot of k' versus $[\mathrm{Cu^{II}(fla)(idpa)}]\mathrm{ClO_4}$ for the above five experiments gave a straight line with a correlation coefficient of 99.29% (Fig. S5).

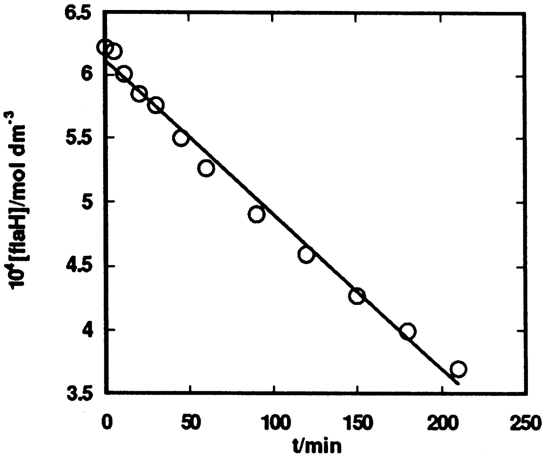


Fig. 4. Time course for the oxygenation of flavonol in DMF (experiment 3, Table 3).

Experiments made at different dioxygen concentrations (calculated from literature data assuming the validity of Dalton's law, the dissolved concentration of $\mathrm{O_2}$ being 6.54×10^{-3} mol dm^{-3} at 130 °C and 760 mmHg $\mathrm{O_2}$ pressure) show hat the reaction is first order with respect to dioxygen concentration (Table 3; experiments 3, 11–14). Plotting k' against $[\mathrm{O_2}]$ for the above five experiments resulted in a straight line with a correlation coefficient of 98.53% (Fig. S6).

According to the kinetic data obtained the oxygenation of flavonol obeys an overall second-order rate equation with $m = 0$, $n = q = 1$ in Eq. (5), from which a mean value of the kinetic constant k_{obs} of $(4.22 \pm 0.15) \times 10^{-2}$ mol^{-2} dm^6 s^{-1} at 403.16 K was obtained (Table 3).

The activation parameters for the catalytic oxygenation reaction were determined from the temperature dependence of the kinetic constant k_{obs}. The Arrhenius plot of $\log k_{\mathrm{obs}}$ versus $1/T$ (Fig. S7) by using the k_{obs} values at 397.16, 403.16, 407.16 and 412.16 K (Table 3; experiments 1–17) was linear with a correlation coefficient of 99.27%. From the slope and the ordinate intercept of this line the parameters $E_a = 75 \pm 6$ kJ mol^{-1}, $\Delta H^{\ddagger} = 71 \pm 6$ kJ mol^{-1} and $\Delta S^{\ddagger} = -97 \pm 15$ J mol^{-1} K^{-1} were calculated.

4. Discussion

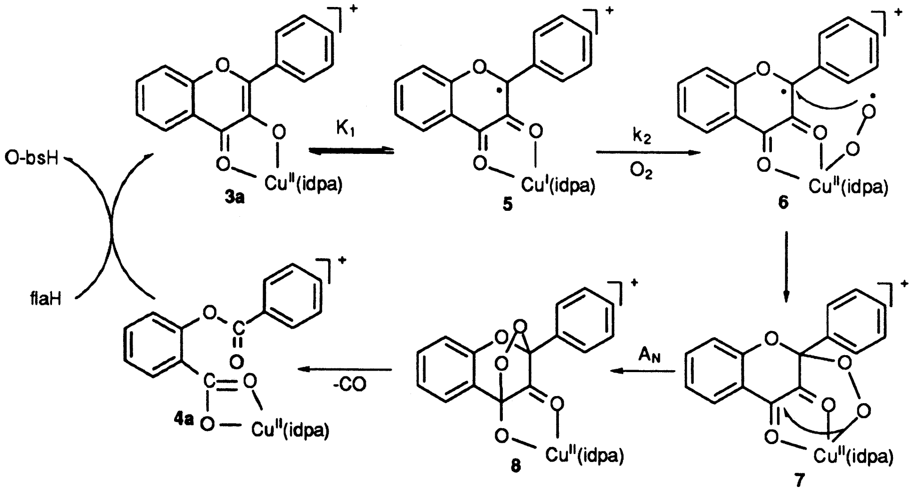
Quercetinase contains Cu(II) ion at the reaction site, but until now there have been only a few mechanistically well-defined chemical precedents in copper chemistry for this enzyme-catalysed reaction. Copper generally functions as a one-electron redox ion, but it may have also multiple roles. Either activation of the substrate, or dioxygen, or both is likely possible. In studies on various metal-containing structural and functional models one concerns whether flavonolate (**1b**) coordinates to the metal centre as a monodentate, or a bidentate ligand. The other concerns the character of flavonol as a ligand, i.e. how flavonol is activated for oxygenation: whether it is just deprotonated to flavonolate or it is also transformed to a flavonoxy radical [12].

We have previously reported that flavonolate as a chelating ligand forms stable complexes with copper ions [15–26]. The crystallographic studies of flavonolato complexes of copper(I) [23] and copper(II) [20,22,25,26] showed the coordination mode of the flavonolate ligand, geometries around the metal ion, and their influence on the delocalisation of π-electrons in the coordinated flavonolate. $[\mathrm{Cu^{II}(fla)_2}]$ [22] and $[\mathrm{Cu^{II}(fla)(idpa)}]\mathrm{ClO_4}$ have been first used as copper(II)-containing model complexes for mimicking the enzyme action. Oxygenation of the above two complexes in DMF solution at elevated temperatures resulted in the corresponding O-benzoylsalicylato copper complexes

Table 4
Comparison of the oxygenation of $[Cu^{II}(4'R\text{–}fla)(idpa)]ClO_4$ and $Cu^{II}(fla)_2$

Complex	$10^2 k_{obs}$ [a] (s^{-1} mol^{-1} dm^3)	$\Delta H^{\ddagger}$ (kJ mol^{-1})	$\Delta S^{\ddagger}$ (J mol^{-1} K^{-1})	ρ
$Cu^{II}(fla)_2$ [b]	0.87 ± 0.15	53 ± 6	-138 ± 11	-0.63
$[Cu^{II}(fla)(idpa)]ClO_4$	0.61 ± 0.02	64 ± 5	-120 ± 13	-0.29

[a] In DMF at 100 °C.
[b] Ref. [22].



Scheme 1.

and carbon monoxide, where the reactions showed simple overall second-order rate expressions. The rate constants (k_{obs}), the activation parameters and the reaction constants ρ of the oxygenation processes are given in Table 4. From the experimental data a mechanism shown in Scheme 1 for the reaction of the flavonolato-copper complex **3a** with dioxygen and the oxygenolysis of flavonol (**1b**) catalysed by $[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) can be proposed.

Flavonolate as a chelating ligand forms stable complexes with copper ions [20–24]. They are stable towards dioxygen in solid form and even in solution at ambient conditions. Compounds **3** are smoothly oxygenated under ambient conditions giving the copper(II) complexes **4** with the new ligand of *O*-benzoylsalicylate formed in the enzyme-like reaction. At elevated temperature the reaction proceeds reasonably faster. Kinetic studies on the oxygenation of the flavonolato complexes **3** established a second-order overall rate expression, indicating that the rate-determining step must be bimolecular. We believe that in a fast preequilibrium the copper(II) flavonolate complex **3** undergoes intramolecular electron transfer from the ligand fla^- to Cu^{II} resulting in the copper(I) flavonoxy radical complex **5**. To date, a radical intermediate has been only observed in the oxygenation of potassium flavonolate to potassium *O*-benzoylsalicylate in aprotic solvents [7]. It is possible that a copper(I) flavonoxy radical is an obligatory intermediate in the reaction but its steady-state concentration is very low, e.g. the preequilibrium between **3** and **5** lies far to the left (K_1 is rather small). The relatively slow reaction rate of the formation of $[Cu^{II}(idpa)(O\text{-}bs)]ClO_4$ (**4**) may support this assumption. The biradical dioxygen may react then at both sites, in an oxidative addition to the copper leading to a superoxocopper complex **6**, or in a radical–radical reaction with the flavonoxy ligand. We believe the former to be the rate-determining step, supported by the kinetic data and mainly by the negative entropy of activation. Reduction of nitroblue tetrazolium (NBT) to blue formazan was used as an indicator of $O_2^{\bullet -}$, and this test indicates the absence of free $O_2^{\bullet -}$, which also supports the formation of a Cu(II)–O_2 complex [34]. This is followed then in a fast consecutive formation of the trioxametallocycle **7**, which reacts by an intramolecular nucleophilic addition on the C4 carbon to give the endoperoxide **8**. The nucleophilic attack of the bound peroxide could, however, also take place on the 3-C=O group as well, giving rise to a 1,2-dioxetane intermediate. The absence of chemiluminescence during the reaction, which is very characteristic for the decomposition of 1,2-dioxetanes to carbonyl compounds, seems to render this pathway unlikely [35]. This may be due to a much less steric strain in the five-membered cyclic peroxide, compared to that in the four-membered 1,2-dioxetane, or may have also an electronic explanation due to higher electron density on the 3-C=O carbon than in the case of 4-C=O carbon accessible by the nucleophilic attack. The endoperoxide then decomposes in a fast step to the *O*-benzoylsalicylato copper complex **4** and carbon monoxide.

Assuming steady-state conditions for species **5**, the rate equation (7) can be deduced

$$-\frac{\mathrm{d[Cu]}}{\mathrm{d}t}=\frac{k_1k_2[\mathrm{Cu}][\mathrm{O_2}]}{k_1+k_{-1}+k_2[\mathrm{O_2}]}=\frac{k_1k_2[\mathrm{Cu}][\mathrm{O_2}]}{k_1+k_{-1}}$$
$$=k_{\mathrm{obs}}[\mathrm{Cu}][\mathrm{O_2}] \qquad (7)$$

which after some simplification ($k_2[O_2] \ll k_1 + k_{-1}$ and [Cu] means the concentration of all Cu-complexes) is in good agreement with an overall second-order dependence according the experimental data obtained in the kinetic measurements.

The reaction rate of the oxygenation of $[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) is somewhat slower than that found for the oxygenation of $[Cu^{II}(fla)_2]$. The differences in the k_{obs} values and the activation parameters (Table 4) can be explained by stabilising and retarding effect of the idpa ligand on the intramolecular electron transfer in the preequilibrium step ($k_1 \gg k_{-1}$).

The bimolecular reaction step is also supported by the Hammett correlation (Fig. 2, Table 2). The magnitude and sign of ρ reflects the geometry of the transition state [36,37] and indicates the influence of the *para* substituents on the remote reaction centre. Since the Hammett plot for all of the substituents results in a straight line, we conclude that there is no change in the mechanism over the series of flavonolato copper complexes **3**. The crucial evidence for a bimolecular reaction step is the reaction constant ρ, which was found to be -0.29. The observation that ρ is moderately large and negative supports the contention that the substituents at the *para*-position have a significant electronic effect at the remote reaction centre. Electron-donating substituents increase the reaction rate, while electron-withdrawing substituents slow down the reaction rate, which is consistent with an electrophilic attack of dioxygen at the copper(I) centre. The reaction constant (ρ) of this reaction is somewhat less (about by half) than that found for the oxygenation of $[Cu^{II}(fla)_2]$ ($\rho = -0.63$). This may be due to a doubled electronic effect of two R-groups on the two flavonolate ligands.

We have found that the oxygenation of flavonol (**1b**) using $[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) as catalyst results in the oxidative cleavage of the heterocyclic ring to give *O*-benzoylsalicylic acid (**2b**) and CO as primary products. Secondary products derived from *O*-benzoylsalicylic acid, such as salicylic acid, benzoic acid, and *N*,*N*-dimethylbenzamide due to hydrolysis and amidation of benzoic acid by DMF, were also formed. The oxidation is selective and no other products were obtained. The results of the kinetic measurements showed an overall second-order rate expression supported by the large negative $\Delta S^{\ddagger}$ value (-97 ± 15 J mol^{-1} K^{-1}). On the other hand, the rate equation suggests that the substrate flavonol does not play a role in the rate-determining step. On the basis of the kinetic data the mechanism shown in Scheme 1 can be proposed for the catalytic oxygenation as well. We believe that the oxygenation of flavonol (**1b**) proceeds in the same way as the stoichiometric oxygenation of $[Cu^{II}(fla)(idpa)]ClO_4$ (**3a**) resulting in the $[Cu^{II}(idpa)(O\text{-bs})]ClO_4$ (**4a**) complex. Owing to a ligand scrambling reaction, flaH displaces *O*-bsH in **4a** where the starting catalyst **3a** and *O*-benzoylsalicylic acid (**2b**) is formed to close the catalytic cycle.

As a conclusion it can be said that the present work provides further support to the key role of copper ion in these oxygenation reactions, which may be regarded as functional models for quercetinase. We believe that the copper ion in the enzyme, and also in model reactions, acts as an electron buffer, due to their ability to accept or donate electrons. In this sense in the activation process the flavonolato ligand is transformed to flavonoxy radical and copper(I). This reacts then with dioxygen to form a peroxidic species, which transforms to the endoperoxide (**8**), and finally to the *O*-benzoylsalicylato copper(II) complex **4a**. The kinetic data obtained are in good agreement with a bimolecular, rate-determining elementary step. The preceded valence tautomerism between **3** and **5** could not be proved due to the instability of the flavonoxy species even in aprotic solvents. Theoretical calculations support that too, indicating the high energy content of the flavonoxy radical [38]. This contrasts the situation found in the case of copper catecholate and copper semiquinone systems, where the orbitals of the copper ion and the ligands involved in the binding matches well in energy resulting in a balanced equilibrium [39]. The steps after the rate-determining step are still speculative, since all efforts to isolate the endoperoxides **8** failed until now. However, our recent works on studies of the influence of the various ligands on the selectivity of the oxygenation reactions of flavonolato(copper) complexes shows that not only the endoperoxides **8** may be formed but also 1,2-dioxetanes depending on the ligand, which could be proved [40]. This makes the assumption of the endoperoxide **8** not unreasonable. The stability of these is probably rather low, which may explain the absence of any data of the core structure of this peroxide. Works are now in progress to clear these points in order to understand better the mechanistic aspects of this interesting enzyme reaction.

5. Supplementary material

Kinetic diagrams for stoichiometric oxygenation of **3** and catalytic oxygenation of **1** is available free of charge via the Internet at http://www.ccdc.cam.ac.uk

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References

[1] (a) F.S. Sariaslani, Crit. Rev. Biotechnol. 9 (1989) 171;
(b) A.E. True, A.M. Orville, L.L. Pearce, J.D. Lipscomb, L. Que Jr., Biochemistry 29 (1990) 10847;
(c) I. Bertini, F. Briganti, S. Mangani, H.F. Nolting, A. Scozzafava, Biochemistry 33 (1994) 10777;
(d) D.M. Arciero, J.D. Lipscomb, J. Biol. Chem. 261 (1986) 2170;
(e) M.R. Harpel, J.D. Lipscomb, J. Biol. Chem. 265 (1990) 6301;
(f) S.A. Wolgel, J.E. Dege, P.E. Perkins-Olson, C.H. Juarez-Garcia, R.L. Crawford, E. Münck, J.D. Lipscomb, J. Bacteriol. 175 (1993) 4414.

[2] (a) D.W.S. Westlake, G. Talbot, E.R. Blakely, F.J. Simpson, Can. J. Microbiol. 5 (1959) 621;
(b) F.J. Simpson, G. Talbot, D.W.S. Westlake, Biochem. Biophys. Res. Commun. 2 (1960) 15;
(c) S. Hattori, I. Noguchi, Nature (London) 184 (1959) 1145.

[3] (a) T. Oka, F.J. Simpson, H.G. Krishnamurty, Can. J. Microbiol. 16 (1977) 493;
(b) H.G. Krishnamurty, F.J. Simpson, J. Biol. Chem. 245 (1970) 1476.

[4] (a) E. Makasheva, N.T. Golovkina, Zh. Obsch. Khim. 43 (1973) 1640;
(b) M. Thomson, C.R. Williams, Anal. Chim. Acta 85 (1976) 375;
(c) K. Takamura, M. Ito, Chem. Pharm. Bull. 25 (1977) 3218.

[5] A. Nishinaga, T. Tojo, H. Tomita, T.J. Matsuura, J. Chem. Soc., Perkin Trans. 1 (1979) 2511.

[6] V. Rajananda, S.B. Brown, Tetrahedron Lett. 22 (1981) 4331.

[7] L. Barhács, J. Kaizer, G. Speier, J. Org. Chem. 39 (2000) 4224.

[8] T. Matsuura, H. Matsushima, R. Nakashima, Tetrahedron Lett. 26 (1970) 435.

[9] M.M.A. El-Sukkary, G. Speier, J. Chem. Soc., Chem. Commun. (1981) 745.

[10] A. Nishinaga, T. Tojo, T. Matsuura, J. Chem. Soc., Chem. Commun. (1974) 896.

[11] A. Nishinaga, N. Numada, K. Maruyama, Tetrahedron Lett. 30 (1989) 2257.

[12] A. Nishinaga, T. Kuwashige, T. Tsutsui, T. Mashino, K. Maruyama, J. Chem. Soc., Dalton Trans. (1994) 805.

[13] M. Utaka, M. Hojo, Y. Fujii, A. Takeda, Chem. Lett. (1984) 635.

[14] M. Utaka, A. Takeda, J. Chem. Soc., Chem. Commun. (1985) 1824.

[15] É. Balogh-Hergovich, G. Speier, J. Mol. Catal. 71 (1992) 1.

[16] G. Speier, V. Fülöp, L. Párkányi, J. Chem. Soc., Chem. Commun. (1990) 512.

[17] É. Balogh-Hergovich, G. Speier, G. Argay, J. Chem. Soc., Chem. Commun. (1991) 551.

[18] I. Lippai, G. Speier, G. Huttner, L. Zsolnai, Chem. Commun. (1997) 741.

[19] I. Lippai, G. Speier, J. Mol. Catal. 130 (1998) 139.

[20] I. Lippai, G. Speier, G. Huttner, L. Zsolnai, Acta Crystallogr., Sect. C 53 (1997) 1547.

[21] É. Balogh-Hergovich, J. Kaizer, G. Speier, Inorg. Chim. Acta 256 (1997) 9.

[22] É. Balogh-Hergovich, J. Kaizer, G. Speier, G. Argay, L. Párkányi, J. Chem. Soc., Dalton Trans. (1999) 3847.

[23] É. Balogh-Hergovich, J. Kaizer, G. Speier, V. Fülöp, L. Párkányi, Inorg. Chem. 38 (1999) 3787.

[24] É. Balogh-Hergovich, J. Kaizer, G. Speier, J. Mol. Catal. 159 (2000) 215.

[25] É. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner, A. Jacobi, Inorg. Chem. 39 (2000) 4224.

[26] É. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner, L. Zsolnai, Inorg. Chim. Acta 304 (2000) 72.

[27] D.F. Shriver, M.A. Drezdzon, The Manipulation of Air-sensitive Compounds, Wiley, New York, 1986.

[28] M.A. Smith, R.M. Newman, R.A. Webb, J. Heterocycl. Chem. 5 (1968) 425.

[29] F. Arndt, in: A.H. Blatt (Ed.), Organic Syntheses, vol. 2, Wiley, New York, 1943, p. 165.

[30] A. Kruis, Landolt–Börnstein, bd. 4, teil 4, Springer, Berlin, 1976, p. 269.

[31] G. Ram, A.R. Sharaf, J. Indian Chem. Soc. 45 (1968) 13.

[32] L. Jurd, in: T.A. Geissman (Ed.), The Chemistry of Flavonoid Compounds, Pergamon, New York, 1962, p. 107.

[33] T.J. Mabry, K.R. Markham, M.B. Thomas, The Systematic Identification of Flavonoids, Springer, Berlin, 1970.

[34] B.H.J. Beilski, H.W. Richter, J. Am. Chem. Soc. 99 (1977) 3019.

[35] M. Schulz, K. Kirschke, in: D. Swern (Ed.), Organic Peroxides, vol. III, Wiley-Interscience, New York, 1972, p. 67.

[36] J.F.J. Dippy, J.E.J. Page, J. Chem. Soc. (1938) 357.

[37] H.H.J. Jaffe, J. Chem. Phys. (1953) 415.

[38] L. Bencze, G. Speier, to be published.

[39] C.G. Pierpont, C.W. Lange, Prog. Inorg. Chem. 41 (1994) 331.

[40] É. Balogh-Hergovich, J. Kaizer, G. Speier, G. Huttner, L. Zsolnai, J. Inorg. Biochem. 67 (1997) 75.