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Kinetics and mechanism of the Cu(I) and Cu(II) flavonolate-catalyzed oxygenation of flavonol. Functional quercetin 2,3-dioxygenase models

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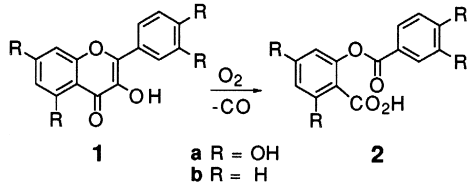
Abstract

The oxygenation of flavonol (flaH) using $Cu^{II}(fla)_2$ and $Cu^{I}(fla)(PPh_3)_2$ catalysts results in oxidative cleavage of the heterocyclic ring to give *O*-benzoylsalicylic acid (*O*-bsH) and CO as primary products. The oxygenolysis of flavonol catalyzed by $Cu^{II}(fla)_2$ in DMF was followed by electronic spectroscopy and the rate law was found to be $-d[flaH]/dt = k_{obs}[flaH][Cu^{II}(fla)_2][O_2]$. The rate constant, activation energy, activation enthalpy and entropy at 393 K are as follows: $k_{obs}/s^{-1}\ mol^{-2}\ dm^{-6} = (2.02 \pm 0.07) \times 10^3$, $E_a/kJ\ mol^{-1} = 142 \pm 6$, $\Delta H^{\ddagger}/kJ\ mol^{-1} = 139 \pm 5$, and $\Delta S^{\ddagger}/J\ mol^{-1}\ K^{-1} = 168 \pm 13$. The results of the kinetic measurements of the $Cu^{I}(fla)(PPh_3)_2$-catalyzed oxygenation shows that in the presence of a large excess of the substrate, the $Cu^{I}(fla)(PPh_3)_2$ reacts with flavonol in an irreversible step to $Cu^{II}(fla)_2$, and, then, the mechanism of the oxygenation is the same as that with the $Cu^{II}(fla)_2$-catalyzed reaction.

Keywords: Copper; Flavonolate complexes; Dioxygenation; Quercetinase

1. Introduction

Biological oxygenations catalyzed by oxygenases are very important processes in nature for the metabolism of various organic substances. Oxygenases are metal-containing proteins and a fair number of them utilizes copper at their active sites [1–5]. One of these metalloenzymes is the copper-containing quercetin 2,3-dioxygenase, which catalyzes the oxygenolysis of 3-hydroxyflavones (**1**) to the corresponding depsides (**2**) as a result of the oxidative cleavage of the heterocyclic ring (Eq. (1)) [6–10].



(1)

Quercetin (**1a**) was assumed to coordinate to copper(II) as a chelating ligand at the 3-hydroxy and 4-carbonyl groups [11–13]. Since oxidation

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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.

Few model reactions on the oxygenation of flavonol (**1b**, flaH) and quercetin (**1a**) have been carried out with the aim to shed light on this curious reaction leading to oxidative ring cleavage of the pyranone ring with concomitant extrusion of carbon monoxide. Base-catalysed oxygenation of quercetin (**1a**) and flavonol (**1b**) under aqueous [14] and non-aqueous conditions [15,16], photosensitized oxygenations [17], and reactions with superoxide anion [18], have been studied. Cobalt [19–21] and copper [22–24] complexes were applied as suitable catalysts for the oxygenation reaction. Copper(I) [25] and copper(II) [26] flavonolate complexes were also found to catalyze the reaction.

As a continuation of our studies on the preparation, characterization and oxygenation of copper flavonolate complexes [27–32], we tried to study their catalytic activity at the oxygenation of flavonol. This paper deals with the kinetics of the oxygenation of flavonol (**1b**) catalyzed by $Cu(fla)_2$ and $Cu(fla)(PPh_3)_2$.

2. Experimental

2.1. Instrumentation

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 ionization detector (CP SIL 8CB column), and a thermal conductivity detector (molecular sieve 5A column). GC–MS measurements were recorded on HP 5890II/5971 GC/MSD at 75 eV.

2.2. Materials and methods

Solvents (diethyl ether and DMF) were purchased from Aldrich in analytically pure quality. Diethyl ether was distilled from K–Na amalgam under Ar atmosphere. DMF was purified by azeotropic distillation with benzene and water and stored under argon in the dark to avoid direct light. Flavonol [14], $Cu^{II}(fla)_2$ [31] and $Cu^{I}(fla)(PPh_3)_2$ [32] were prepared by literature methods. Diazomethane [33] 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 P_2O_5 and Blaugel in order to remove traces of water and other impurities.

2.3. Catalytic oxygenation of flavonol catalyzed by $Cu^{II}(fla)_2$

A total of 0.119 g (0.5 mmol) flavonol and 0.054 g (0.1 mmol) $Cu^{II}(fla)_2$ were dissolved and stirred at 80°C in 20 cm^3 DMF for 10 h under dioxygen atmosphere. The formation of *O*-benzoylsalicylic acid from flavonol requires dioxygen, but no apparent oxygen uptake was observed, because the absorption of oxygen and the liberation of carbon monoxide compensate each other. The GC analysis of the gas phase showed 0.37 mmol CO (73% conversion). Approximately 0.37 mmol of dioxygen has been consumed and as much carbon monoxide has been evolved during the reaction. A 2-cm^3 diazomethane solution (in diethyl ether) was added to 1 cm^3 of the reaction mixture at room temperature and the conversion of the flavonol (78%) and the yields of the products *N,N*-dimethylbenzamide (8%), benzoic acid (14%), salicylic acid (23%) and *O*-benzoylsalicylic acid (55%) were determined by GC.

2.4. Catalytic oxygenation of flavonol catalyzed by $Cu^{I}(fla)(PPh_3)_2$

A total of 0.119 g (0.5 mmol) flavonol and 0.083 g (0.1 mmol) $Cu^{I}(fla)(PPh_3)_2$ was dissolved and stirred at 80°C in 20 cm^3 DMF for 10 h under dioxygen atmosphere. The GC anal-

Table 1
Kinetic data for the $Cu^{II}(fla)_2$-catalyzed oxygenation of flaH in DMF solution

Experiment no.	Temperature (°C)	$10^3[O_2]$ (mol dm^{-3})	10^4[Cu][a] (mol dm^{-3})	10^4[flaH][a] (mol dm^{-3})	$10^4k'$ (s^{-1})	R[b] (%)	$10^{-3}k_{obs}$ (s^{-1} mol^{-2} dm^{-6})	10^7d[flaH]/dt (mol dm^{-3}s^{-1})
1	120	6.07	0.997	1.64	5.83	97.63	0.96 ± 0.09	0.957
2	120	6.07	0.997	5.00	13.71	99.66	2.27 ± 0.06	6.856
3	120	6.07	0.997	6.81	12.24	99.13	2.02 ± 0.08	8.335
4	120	6.07	0.997	9.50	13.47	99.08	2.23 ± 0.09	12.80
5	120	6.07	0.997	12.00	14.17	99.50	2.34 ± 0.07	17.01
6	120	6.07	0.230	6.81	2.72	99.87	1.95 ± 0.05	
7	120	6.07	0.593	6.81	7.55	98.90	2.10 ± 0.11	
8	120	6.07	1.424	6.81	16.52	99.12	1.91 ± 0.08	
9	120	1.21	0.997	6.81	2.49	99.67	2.06 ± 0.05	
10	120	7.63	0.997	6.81	13.74	99.30	1.81 ± 0.08	
11	120	8.28	0.997	6.81	16.61	99.74	2.01 ± 0.05	
12	120	10.13	0.997	6.81	20.68	99.99	2.05 ± 0.03	
							2.02 ± 0.07[c]	
13	90	7.84	1.191	6.81	0.502	99.92	0.054 ± 0.001	
14	100	7.70	1.187	6.81	1.99	99.45	0.220 ± 0.008	

[a] In 50 cm^3 DMF.
[b] Correlation coefficients of least-squares regressions.
[c] Mean value of the kinetic constant k and its standard derivation $\sigma(k)$ 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 $w_i = 1/\sigma_i^2$.

ysis of the gas phase showed 0.33 mmol CO (65% conversion). A 2-cm^3 diazomethane solution (in diethyl ether) was added to 1 cm^3 of the reaction mixture at room temperature and the conversion of the flavonol (68%) and the yields of the products *N*,*N*-dimethylbenzamide (24%), benzoic acid (20%), salicylic acid (40%) and *O*-benzoylsalicylic acid (16%) were determined by GC.

2.5. Kinetic measurements

Reactions of flavonol with O_2 catalyzed by $Cu^{II}(fla)_2$ and $(Cu^{I}(fla)(PPh_3)_2)$ were per-

Table 2
Kinetic data for the $Cu^{I}(fla)(PPh_3)_2$-catalyzed oxygenation of flaH in DMF solution

Experiment no.	Temperature (°C)	$10^3[O_2]$ (mol dm^{-3})	10^4[Cu][a] (mol dm^{-3})	10^3[flaH][a] (mol dm^{-3})	$10^5k'$ (s^{-1})	R[b] (%)	$10^{-2}k_{obs}$ (s^{-1} mol^{-2} dm^{-6})
1	135	5.88	0.50	1.24	3.025	99.86	1.03 ± 0.02
2	135	5.88	0.98	1.24	5.556	99.76	0.96 ± 0.03
3	135	5.88	1.96	1.24	6.759	99.67	0.59 ± 0.02
4	135	5.88	3.00	1.24	10.35	99.08	0.59 ± 0.04
5	135	5.88	3.92	1.24	13.91	99.55	0.60 ± 0.02
6	135	1.18	1.96	1.24	3.119	99.87	1.35 ± 0.03
7	135	7.01	1.96	1.24	9.604	99.66	0.74 ± 0.02
8	135	13.98	1.96	1.24	20.19	99.42	0.70 ± 0.03
							0.80 ± 0.09[c]
9	140	4.35	1.96	1.24	12.32	99.42	1.69 ± 0.07
10	145	2.25	1.96	1.24	14.38	99.82	2.80 ± 0.07

[a] In 50 cm^3 DMF.
[b] Correlation coefficients of least-squares regressions.
[c] Mean value of the kinetic constant k and its standard derivation $\sigma(k)$ 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 $w_i = 1/\sigma_i^2$.

formed in DMF solutions. In a typical experiment, flavonol and $Cu^{II}(fla)_2$ ($Cu^{I}(fla)(PPh_3)_2$) were dissolved under argon atmosphere in a thermostated 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 flavonol, $Cu^{II}(fla)_2$ or $Cu^{I}(fla)(PPh_3)_2$ were determined by UV-Vis spectroscopy measuring the absorbance of the reaction mixture at 342.5 nm ($\log \varepsilon = 4.24$) [λ_{max} of a typical band of flavonol] and 433 and 430.5 nm ($\log \varepsilon = 4.45$, $\log \varepsilon = 3.89$) [λ_{max} of a typical band of $Cu^{II}(fla)_2$ and $Cu^{I}(fla)(PPh_3)_2$]. The argon was then replaced by dioxygen, and the consumption of flavonol was analyzed periodically (ca. every 10 min). The products did not show absorption in this region. The rate of oxygenation was independent of the stirring rate, excluding eventual diffusion control effects. Experimental conditions are summarized in Tables 1 and 2. The temperature was determined with an accuracy of ± 0.5°C; the pressure of dioxygen was determined with an accuracy of ± 0.5%. The O_2 concentration was calculated from literature data [34] taking into account the partial pressure of DMF [35] and assuming the validity of Dalton's law.

3. Results and discussion

Since it has been assumed for the enzymatic reaction of quercetin 2,3-dioxygenase that quercetin binds to copper(II) through its 3-hydroxy and 4-keto groups, and structural model studies with simple copper compounds and flavonol evidenced this type of coordination, we attempted as a key model reaction the oxygenation of flavonol by the copper flavonolate complexes $Cu^{II}(fla)_2$ and $Cu^{I}(fla)(PPh_3)_2$. The oxygenation did not proceed fast enough in acetonitrile at room temperature. In DMF, however, at elevated temperature, the reaction progressed well, and the disappearance of the flavonol and the concentration of the catalysts could be monitored conveniently by electronic spectroscopy. We found that the oxygenation of flavonol using $Cu^{II}(fla)_2$ and $Cu^{I}(fla)(PPh_3)_2$ catalysts results in oxidative cleavage of the heterocyclic ring to give *O*-benzoylsalicylic acid (*O*-bsH) 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, no other products were obtained. Different products were obtained in the oxidation of flavonol catalyzed by CuCl and $CuCl_2$ [23,24].

3.1. Oxygenation of flavonol catalyzed by $Cu^{II}(fla)_2$

The reactions between flavonol and O_2 in the presence of catalytic amounts of $Cu^{II}(fla)_2$ were performed in DMF solutions and examined in the temperature range from 90°C to 120°C with a ratio between initial concentrations of $Cu^{II}(fla)_2$ and flavonol from 1:2 to 1:30. Experiments were also carried out at different dioxygen concentrations. Experimental conditions are summarized in Table 1.

$Cu^{II}(fla)_2$ exhibits absorption at 433 nm [31]. Flavonol shows an absorption band at 342.5 nm [36,37]. Spectral changes accompanying addition of dioxygen to the DMF solution show that the absorption peak at 342.5 nm decrease, while that at 433 nm increase with time. Plots of the time dependence of the absorption of $Cu^{II}(fla)_2$ and flavonol (Fig. 1; Table 1, experiment 9) show two segments, indicating that there are at least two processes occurring consecutively. The initial gradient (part a) hints to a slower process, while the second one (part b) 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 end-product.

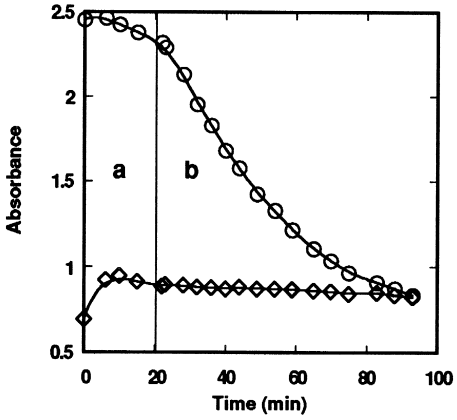


Fig. 1. Spectral changes accompanying the oxygenation of flaH at 342.5 (flaH) (○) and 433 nm ($Cu^{II}(fla)_2$) (◇) during experiment 9 in Table 1.

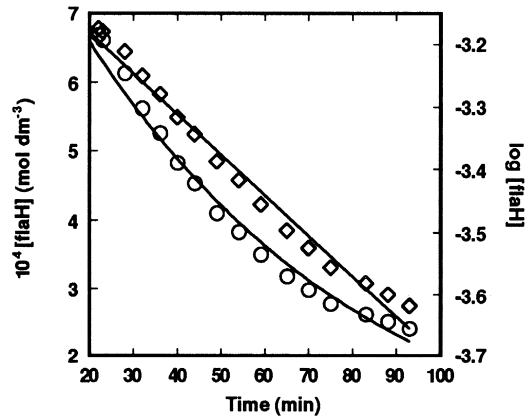


Fig. 2. The time course for the oxygenation of flaH (○) and plot of log[flaH] (◇) vs. time for the oxygenation of flaH (experiment 9, Table 1).

A simple rate law for the catalytic reaction between O_2 and flavonol catalyzed by $Cu^{II}(fla)_2$ is given in Eq. (2).

$$\mathrm{d}[\mathrm{O-bs}]/\mathrm{d}t = -\mathrm{d}[\mathrm{flaH}]/\mathrm{d}t = k\left[\mathrm{Cu^{II}(fla)_2}\right]^m[\mathrm{flaH}]^n[\mathrm{O_2}]^q \quad (2)$$

In order to determine the rate dependence on the various reactants, oxygenation runs were performed at different substrate (Table 1, experiments 1–5), catalyst (Table 1, experiments 3, 6–8) and oxygen concentrations (Table 1, experiments 3, 9–12) under pseudo-first-order conditions. Eq. (2) can then have a simpler feature as written in Eq. (3), k' being the pseudo-first-order rate constant (Eq. (4)).

$$\mathrm{d}[\mathrm{O-bs}]/\mathrm{d}t = -\mathrm{d}[\mathrm{flaH}]/\mathrm{d}t = k'[\mathrm{flaH}]^n \quad (3)$$

$$k' = k_{\mathrm{obs}}\left[\mathrm{Cu^{II}(fla)_2}\right]^m[\mathrm{O_2}]^q \quad (4)$$

From the time dependence of the change of concentration of [flaH] during the oxygenation, the plots of log[flaH] vs. time were linear in experiments 1–5, 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 least-squares method for these linear regressions. A typical first-order plot is shown in Fig. 2 for experiment 9. From variations of the reactions rates, plots of $-\mathrm{d}[\mathrm{flaH}]/\mathrm{d}t$ vs. $[\mathrm{flaH}]_o$ (Fig. 3) were also linear ($R = 99.56\%$) in experiments 1–5, reinforcing that the reaction is indeed first-order with respect to substrate concentration.

Kinetic measurements of the rate with respect to catalyst concentration (Table 1, experiments 3, 6–8) indicate a first-order dependence. A plot of k' vs. $[\mathrm{Cu^{II}(fla)_2}]_o$ for the above four experiments gave a straight line with a correlation coefficient of 99.86% (Fig. 4).

Experiments made at different dioxygen concentrations (calculated from literature data as-

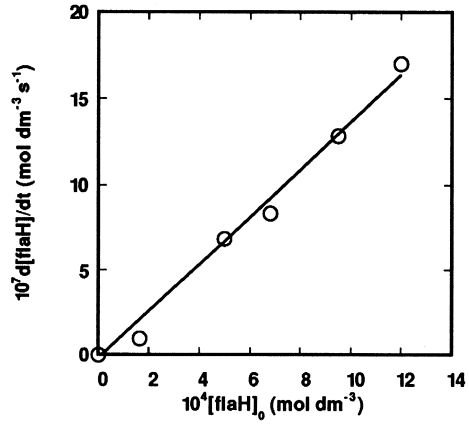


Fig. 3. Plot of oxygenation rate of flaH vs. the initial flaH concentration (experiments 1–5, Table 1).

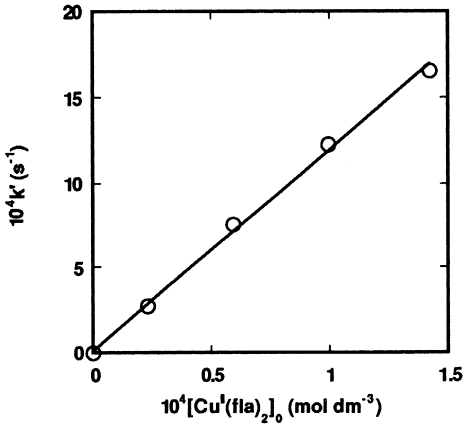


Fig. 4. Plot of pseudo-first-order reaction rate constant (k') vs. the initial $Cu^{II}(fla)_2$ concentration for the oxygenation of flavonol (experiments 3, 6–8, Table 1).

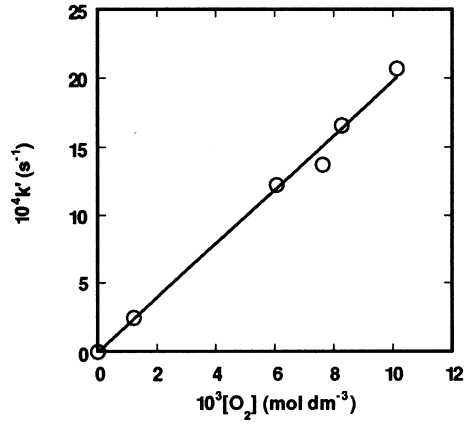


Fig. 5. Plot of pseudo-first-order reaction rate constant (k') vs. the O_2 concentration for the oxygenation of flavonol (experiments 3, 9–12, Table 1).

suming the validity of Dalton's law, the dissolved concentration of O_2 being 6.07×10^{-3} mol dm^{-3} at 120°C and 760 mm Hg O_2 pressure) show that the dioxygen concentration affects the rate of the reactions (experiments 3, 9–12; columns k' and R in Table 1) and that the reaction is first-order with respect to dioxygen concentration. A plot of k' vs. $[O_2]$ for the above five experiments gave a straight line with a correlation coefficient of 99.64% (Fig. 5).

According to the kinetic data obtained, the oxygenation of flaH obeys an overall third-order rate equation with $m = n = q = 1$ in Eq. (2), from which a mean value of the kinetic constant k_{obs} of $(2.02 \pm 0.07) \times 10^3$ s^{-1} mol^{-2} dm^{-6} at 393 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 Arrhenius plot of $\log(k_{obs})$ vs. $1/T$ (Fig. 6), by using the k_{obs} values at 363, 373 and 393 K (experiments 3, 13–14 in Table 1), was linear with a correlation coefficient of 99.91%. The slope and the ordinate intercept of this line gave $E_a = 142 \pm 6$ kJ mol^{-1}, $\Delta H^{\ddagger} = 139 \pm 5$ kJ mol^{-1} and $\Delta S^{\ddagger} = 168 \pm 13$ J mol^{-1} K^{-1}.

On the basis of the experimental data, the following mechanism for the catalytic oxygenation of flavonol can be proposed (Scheme 1). Oxygenation of $Cu^{II}(fla)_2$ in DMF solution at ambient and somewhat elevated temperatures gives $Cu^{II}(O\text{-bs})_2$ and carbon monoxide and the reaction showed simple overall second-order rate expression [31]. We believe that in the presence of a large excess of flavonol, the first step is the formation of the addition complex $H[Cu^{II}(fla)_3]$ (**3**) in a equilibrium process (K_1). Thereafter the complex $H[Cu^{II}(fla)_3]$ (**3**) undergoes in a fast preequilibrium an intramolecular electron transfer from the ligand fla^- to Cu^{II} resulting in the copper(I) flavonoxyl radical complex

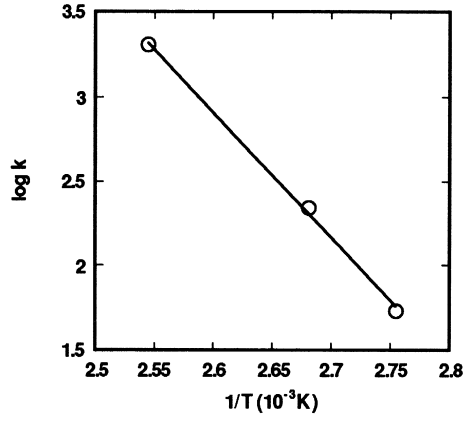
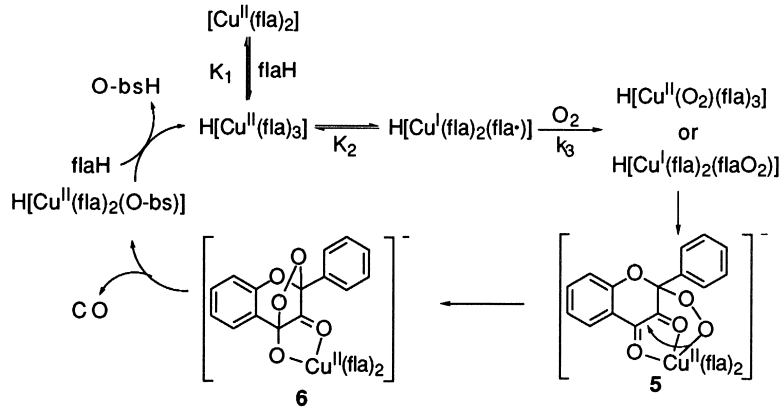
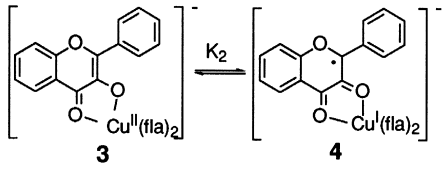


Fig. 6. Arrhenius plot of rate constant, k', for the oxygenation of flaH.



Scheme 1.

$H[Cu^{I}(fla)_2(fla^{\cdot})]$ (**4**) as shown in Eq. (5). This equilibrium



(5)

is also largely shifted to the left (K_2 is rather small). The flavonoxyl radical is not persistent under the conditions as have been found at the oxygenation of Kfla [16], and there was no evidence for the formation of the dehydro dimer of flavonol, which may be the result of the radical coupling of $fla^{\cdot}$ ligands. In the copper(I) flavonoxyl radical complex (**4**), there are two redox-active centers, the radical ligand $fla^{\cdot}$ and the copper(I) ion. The biradical dioxygen may react at both sites, in an oxidative addition to copper(I) giving a superoxocopper(II) complex $H[Cu^{II}(O_2)(fla)_3]$, or in a radical–radical reaction with the flavonoxyl ligand leading to $H[Cu^{I}(fla)_2(flaO_2^{\cdot})]$. We believe the latter to be the rate-determining step since in $H[Cu^{I}(fla)_2(fla^{\cdot})]$, the copper(I) is coordinatedly saturated by the fla^{-} and $fla^{\cdot}$ ligands, and the $fla^{\cdot}$ ligand is more easily accessible by O_2. The peroxyl radical and the copper(I) center cycles in a fast reaction to the trioxametallocycle (**5**), which facilitates a nucleophilic attack of the bound peroxide on the 4C=O group to yield the endoperoxide (**6**). Then, the endoperoxide decomposes in a fast step to the *O*-benzoylsalicylato copper complex and carbon monoxide. Then, flavonol replaces the *O*-benzoylsalicylato ligand again in a fast reaction and so closes the catalytic cycle. The activation parameters of the oxygenation reaction based on an observed reaction constant k_{obs} should be considered with caution, since they contain the preequilibrium constants K_1 and K_2 together with the k_3 value, assumed as the rate-determining step.

3.2. Oxygenation of flavonol catalyzed by $Cu^{I}(fla)(PPh_3)_2$

The reactions between flavonol and O_2 in the presence of catalytic amounts of $Cu^{I}(fla)(PPh_3)_2$ were performed in DMF solutions and examined in the temperature range from 135°C to 145°C with a ratio between initial concentrations of $Cu^{I}(fla)(PPh_3)_2$ and flavonol from 1:3 to 1:25. Experiments were also carried out at different dioxygen concentrations. Experimental conditions are summarized in Table 2.

The complex $Cu^{I}(fla)(PPh_3)_2$ exhibits absorption at 430.5 nm [32], and flavonol shows an absorption band at 342.5 nm in the UV-Vis spectrum. Spectral changes accompanying addition of dioxygen to the DMF solution show that the absorption peak at 342.5 nm decreases, while

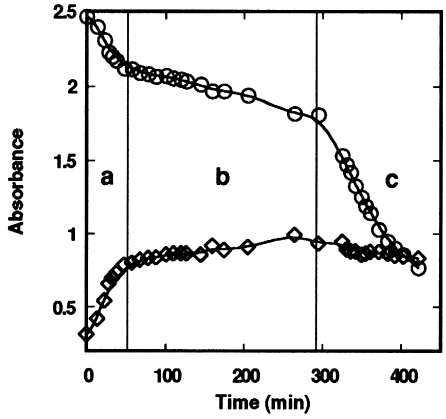


Fig. 7. Spectral changes accompanying the oxygenation of flaH at 342.5 (flaH) (o) and 430.5 nm ($Cu^{I}(fla)(PPh_3)_2$) (◇) during experiment 5 in Table 2.

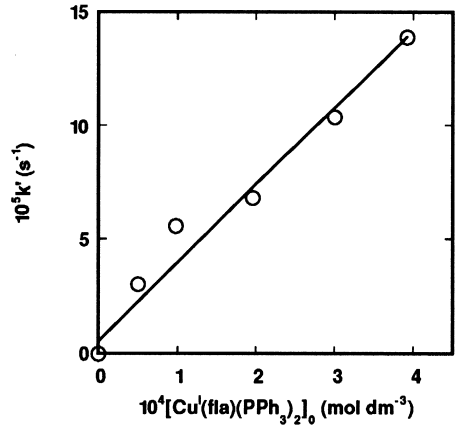


Fig. 9. Plot of pseudo-first-order reaction rate constant (k') vs. the initial $Cu^{I}(fla)(PPh_3)_2$ concentration for the oxygenation of flavonol (experiments 1–5, Table 2).

that at 430.5 nm increases with time. Plotting the time dependence of the absorption of $Cu^{I}(fla)(PPh_3)_2$ and flavonol (experiment 5 in Table 2) shows three segments (Fig. 7), indicating that there are at least three processes occurring consecutively. The initial two gradients hint to slower processes, while the third one may be considered as the actual oxygenation process of the coordinated flavonolate ligand. The first and second one (parts a and b) is believed to correspond to a slow conversion of the complex to another one, which then reacts with dioxygen to the end-product (part c).

In the presence of an excess in flavonol and constant dioxygen pressure, the decrease of the flavonol concentration (part c) takes the course as shown in Fig. 8. Plotting the logarithm of the flavonol concentration against the time, a straight line was obtained, indicating that the rate of the reaction is first-order with respect to the substrate. The rate of oxygenation of flavonol at different catalyst concentrations (experiments 1–5; columns k' and R in Table 2) and at various dioxygen pressures (assuming the validity of Dalton's law) (experiments 5–8; columns

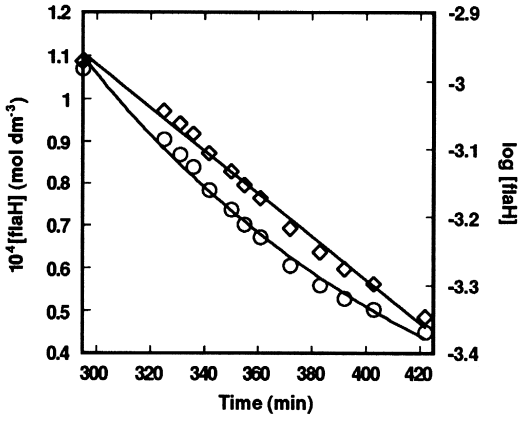


Fig. 8. The time course for the oxygenation of flaH (○) and plot of log[flaH] (◇) vs. time for the oxygenation of flaH (experiment 5, Table 2).

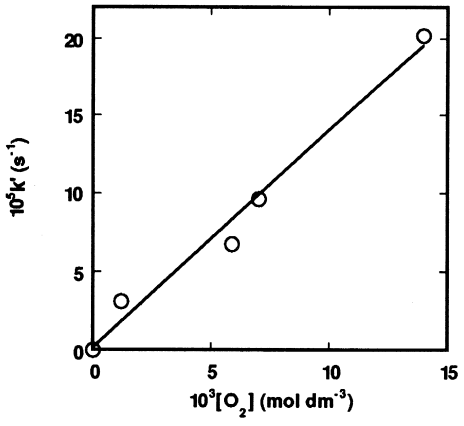


Fig. 10. Plot of pseudo-first-order reaction rate constant (k') vs. the O_2 concentration for the oxygenation of flavonol (experiments 5–8, Table 2).

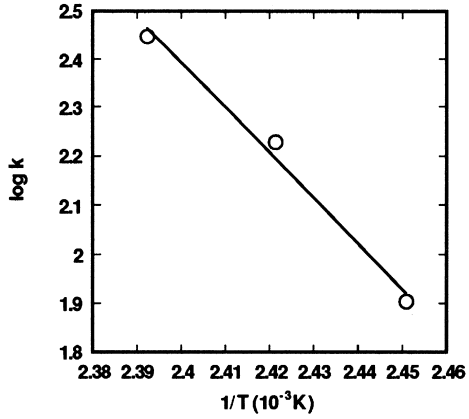


Fig. 11. Arrhenius plot of rate constant, k', for the oxygenation of flaH.

k' and R in Table 2) was also determined. Plotting the pseudo-first-order reaction rate constant of the oxygenation vs. the catalyst concentration, a straight line ($R = 98.55\%$) was obtained (Fig. 9), and, similarly, by plotting the pseudo-first-order reaction rate constant against $[O_2]$ again, a straight line ($R = 99.02\%$) was established (Fig. 10), indicating that the reaction order with respect to the catalyst and dioxygen is one. The rate expression therefore has the form (Eq. (6)):

$$\begin{aligned} &\mathrm{d}[\text{O-bs}]/\mathrm{d}t \\ &\quad = -\mathrm{d}[\text{flaH}]/\mathrm{d}t \\ &\quad = k_{\text{obs}}\left[\mathrm{Cu^{I}(fla)(PPh_3)_2}\right][\text{flaH}][\mathrm{O_2}] \end{aligned} \qquad (6)$$

The value of k_{obs} is calculated as $(0.80 \pm 0.09) \times 10^2$ $\mathrm{s^{-1}\ mol^{-2}\ dm^{-6}}$ at 408 K. The activation parameters for the oxygenation reaction were determined from the temperature dependence of the kinetic constant k_{obs}. The Arrhenius plot of $\log(k_{\text{obs}})$ vs. $1/T$ by using the k_{obs} values at 408, 413 and 418 K (experiments 1–10 in Table 2; Fig. 11) is linear with a correlation coefficient of 99.44%. The slope and the ordinate intercept of this line gives $E_a = 178 \pm 7$ kJ $\mathrm{mol^{-1}}$, $\Delta H^{\ddagger} = 175 \pm 6$ kJ $\mathrm{mol^{-1}}$ and $\Delta S^{\ddagger} = 211 \pm 15$ J $\mathrm{mol^{-1}\ K^{-1}}$.

From the experimental data, a mechanism for the catalytic oxygenation of flavonol can be proposed. Oxygenation of $\mathrm{Cu^{I}(fla)(PPh_3)_2}$ in DMF solution at ambient conditions gives $\mathrm{Cu^{I}}(O\text{-bs})\mathrm{PPh_3)_2}$ and carbon monoxide, where the reaction showed a simple overall second-order rate expression [32]. It is interesting to note that copper(I) is not oxidized to copper(II) even under more severe conditions. We believe that in the presence of a large excess of the substrate complex $\mathrm{Cu^{I}(fla)PPh_3)_2}$ reacts with flavonol in an irreversible step to $\mathrm{Cu^{II}(fla)_2}$ as indicated in Eq. (7) (sections a and b in Fig. 8) and then the oxygenation of flavonol proceeds in the same way as with the $\mathrm{Cu^{II}(fla)_2}$-catalyzed reaction. When we added triphenylphosphine to the reaction mixture at the oxygenation of flavonol catalyzed by $\mathrm{Cu^{II}(fla)_2}$ also lower reaction rates were obtained.

$$\begin{aligned} &2\mathrm{Cu^{I}(fla)(PPh_3)_2} + 2\text{flaH} + 2.5\mathrm{O_2} \\ &\quad = 2\mathrm{Cu^{II}(fla)_2} + 4\mathrm{O} = \mathrm{PPh_3} + \mathrm{H_2O} \end{aligned} \qquad (7)$$

The somewhat lower reaction rate of the $\mathrm{Cu^{I}(fla)PPh_3)_2}$-catalyzed oxygenation of flavonol and the differences on the activation parameters can be explained by the stabilizing and retarding effect of triphenylphosphine in one of the steps before or in the rate-determining step.

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