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Review

Redox and nonredox metal assisted model systems with relevance to flavonol and 3-hydroxyquinolin-4(1*H*)-one 2,4-dioxygenase

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Contents

Abstract

This review deals with copper and potassium complexes derived from flavonol and 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline and covers various aspects, synthesis, spectroscopic features and chemical reactivity of these compounds. Application of these complexes as structural and their oxygenation reactions as biomimetic functional models with relevance to flavonol and 3-hydroxyquinolin-4(1*H*)-one 2,4-dioxygenases are briefly described in the respected sections.

Keywords: Bioinorganic chemistry; CO-releasing dioxygenases; Flavonol; 4-Oxoquinolines; Biomimetic oxygenation

Abbreviations: PhquinH$_2$, 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline; flaH, flavonol; *O*-bsH, *O*-benzoylsalicylic acid; *N*-baaH, *N*-benzoylanthranilic acid; PPh$_3$, triphenylphosphine; tmeda, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; py, pyridine; DMSO, dimethylsulfoxide; DMF, dimethylformamide; SET, single electron transfer; DPPH, 2,2-diphenyl-1-picrylhydrazyl; TEMPO, 2,2,6,6-tetramethyl-1-piperidinyloxyl

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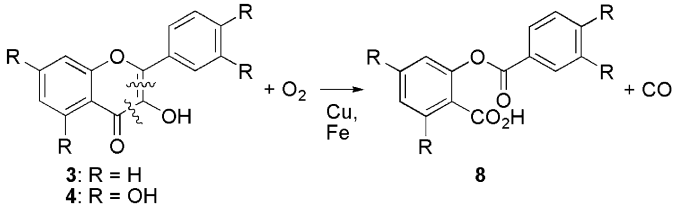
1. Introduction

Biological oxygenations catalyzed by oxygenases are very important processes in nature for the metabolism of various organic substances [1–8]. Oxygenases are a class of enzymes that catalyze the incorporation of oxygen atoms from dioxygen into a substrate. Depending on whether an oxygenase catalyzes the insertion of one or both atoms of dioxygen into its substrate, it is named monooxygenase or dioxygenase. *N*- and *O*-heterocyclic compounds are broadly spread in nature [9–12]. Among them there are a large number of benzopyrone derivatives (**1**, e.g., flavonoids) in plants and the isoelectronic *N*-compounds, the 4-quinolones (**2**) are also found in coal tar and crude oil, etc. From the benzopyrone derivatives the flavonoids (**3**,**4**) and from the quinolones the 3-hydroxy-2-alkyl derivatives (**5**,**6**) of **2** desire attention (Scheme 1).

The former exhibits a large array of biological activities such as antiinflammatory [13] and anticarcinogenic [14] behavior. This may be due to their ability to bind metal ions and/or undergo oxidation by O_2. This may involve either the catecholate moiety of **4** (C ring) or the 3-hydroxy and 4-one groups (B ring). On the other hand, the oxidative metabolism of these heterocycles may be interesting in order to better understand the biological role of both classes of compounds on the one side and the mechanistic aspects of oxidative degradation on the other.

Carbon monoxide forming enzymes are rare. To date, four prokaryotic dioxygenases are known, which catalyze oxidative C–C bond cleavage with incorporation of two O-atoms into, and release of CO from the substrate flavonol (**3**) (flavonol 2,4-dioxygenase) [15–20], 3-hydroxyquinaldin-4(1*H*)-one 2,4-dioxygenase, 3-hydroxyquinolin-4(1*H*)-one 2,4-dioxygenase [21,22], and 1,2-dihydroxy-5-(methylthio)pent-1-en-3-one anion 1,3-dioxygenase [23,24].

Oxygenases usually employ a transition metal, or an organic cofactor, to mediate dioxygen activation. Iron and copper are the metals most commonly used because, in their lower oxidation states, they can form complexes with dioxygen, organic substrate, or both, and affect the electronic structure of the bound compound to alter its reactivity. Flavonol 2,4-dioxygenase (FDO) facilitates the incorporation of dioxygen into quercetin (**4**), thereby cleaving its heterocyclic ring to produce the corresponding depside (**8**) (phenolic carboxylic acid ester) and carbon monoxide (Eq. (1)):

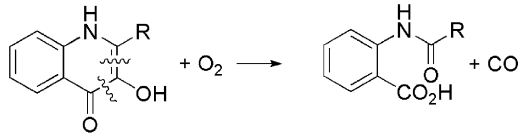


(1)

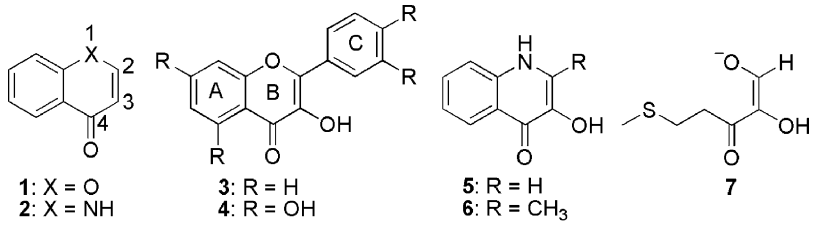
Flavonol 2,4-dioxgenase (also known as quercetin 2,3-dioxygenase or quercetinase) from *Aspergillus flavus* had already been purified in the 1970s and was shown to be a 112 kDa non-blue type 2 copper glycoprotein [25]. The enzyme from *Aspergillus niger* was characterized as a 148 kDa glycoprotein composed of three non-identical subunits; its EPR spectrum showed the characteristic parameters of a type 2 Cu(II) protein [16]. A recent crystal structure determination of flavonol 2,4-dioxygenase from *Aspergillus japonicus* reveals that it forms homodimers. The mononuclear type 2 copper centers displays two distinct geometries: a distorted tetrahedral coordination, formed by three histidine residues and one water molecule, and a distorted trigonal-bipyramidal environment, which additionally comprises a glutamate. The co-existence of these two conformations of the native protein (enzyme) is also present in the native EPR spectrum, which exhibits a mixed signal [26–29].

Further studies have characterized the protein *Yxag* as an iron-containing flavonol 2,4-dioxygenase too. The crystal structure of *Yxag* from *Bacillus subtilis* reveals a dimeric enzyme with two active sites per monomer, each containing a Fe(II) ion [30,31] similar to that of the flavonol 2,4-dioxygenase from *A. japonicus*.

Contrary to the common belief that oxygenases require metal ions and/or organic cofactors for catalysis, several enzymes have been reported recently to catalyze dioxygen incorporation without any apparent requirement for cofactors or metal ions [32,33]. The 2,4-dioxygenolytic ring cleavage of 1*H*-3-hydroxy-4-oxoquinoline and 1*H*-3-hydroxy-4-oxoquinaldine is a key step in the anthranilate pathway of bacterial 4-quinolone and quinaldine degradation, respectively (Eq. (2)) [34,35]:



(2)



Scheme 1.

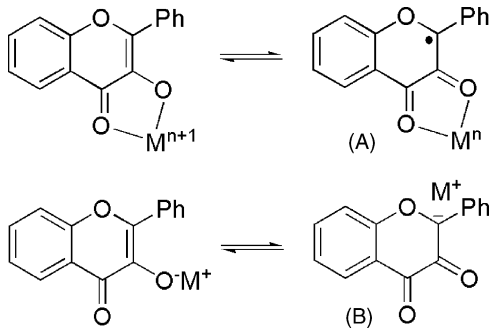


Fig. 1. Probable modes of coordination of flavonol in a flavonol–metal binary intermediate.

Biochemical and spectroscopic studies of the purified 2,4-dioxygenases termed Qdo (1*H*-3-hydroxy-4-oxo-quinoline 2,4-dioxygenase, a 30 kDa monomer) and Hod (1*H*-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase, a 32 kDa monomer) have shown that neither enzyme contains any metal or organic cofactor. We do not know at present the reason why in one case a metal ion is needed and in the other not.

In previous studies the intermediate formation of binary (ES) and ternary species (ESO_2) has been proposed for the oxygenation by flavonol 2,4-dioxygenases as shown in Eq. (3):

$$E \overset{S}{\rightleftharpoons} ES \overset{O_2}{\rightleftharpoons} ESO_2 \rightarrow E + SO_2 \quad (3)$$

Many reports have appeared suggesting the formation and structures of ES from both enzymes and model studies, but until now there has been little mechanistic evidence for the formation of ESO_2 species. There are two types of discussions on the structure of ES. One concerns whether flavonol coordinates to the metallic center as a mono or bidentate ligand or dissociates from the metal ion forming free flavonolate ion in the activation step [36–42]. 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).

Cofactor-less oxygenases present the mechanistically intriguing problem of how triplet dioxygen is activated to react with a singlet organic compound. Formation of a protein radical and a substrate-derived radical (**A**), or direct electron transfer from a deprotonated substrate to molecular oxygen (**B**) to form a caged radical pair may be discussed as hypothetical mechanisms (Fig. 2).

2. Synthesis of copper flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complexes

Flavonol 2,4-dioxygenase is the only firmly established copper dioxygenase known so far. Although the crystal structures of FDO in complex with kaempferol and with the natural substrate quercetin [43] have revealed how substrates are bound in the active site and how the ES complex is ready to be attacked by molecular oxygen in the literature no well-characterized model compounds have been described. In order to obtain more information of the binding of the substrate and to use them in the oxygenation reaction to study the oxidative ring splitting of the heterocyclic ring in the substrate, we first attempted to prepare stable copper(I) and copper(II) flavonolate complexes with different auxiliary ligands. 1*H*-2-Phenyl-3-hydroxy-4-oxoquinoline (**9**) is isoelectronic with flavonol (**3**) and both compounds are degraded by microorganisms by the use of molecular oxygen. In both cases the C^2–C^3 bond in the heterocyclic ring is cleaved by the dioxygenases with concomitant release of carbon monoxide. Since 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**9**) has a nitrogen atom in position 1 of the heterocyclic ring it can also act as a binding site to metal ions. First of all we wish to show that 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline coordinates to copper(II) in a similar fashion as flavonol and if dioxygen is present a facile oxidative cleavage of the C^2–C^3 bond can be observed which resembles the enzymatic reaction.

2.1. Preparation of copper(I) flavonolate complex [44–46]

Since it is well known that Cu(I) is relatively soft and favors soft ligands such as phosphines we tried to prepare copper(I) flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complexes with triphenylphosphine.

Earlier studies have shown that 1,2-diketones react with metallic copper (Cu^0) to give copper(I) semidione species due to electron transfer from Cu^0 to the ketones [47]. Because of the keto–enol tautomerism of 3-hydroxyflavones [48] the keto form **13** does exist in a small concentration and its reaction with Cu^0 in the presence of PPh_3 led to the radical species **14**. It could not be isolated in pure form because of the not negligible

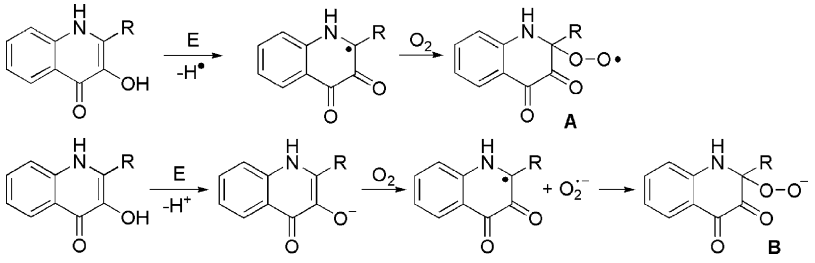
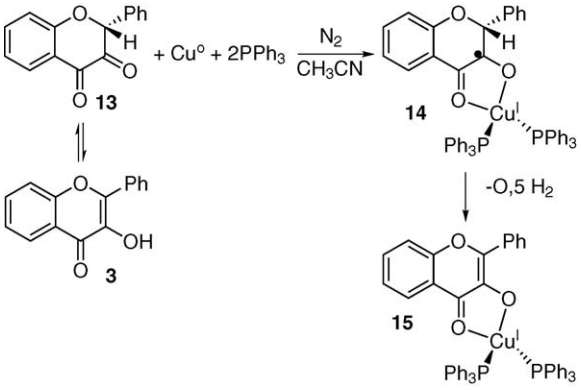


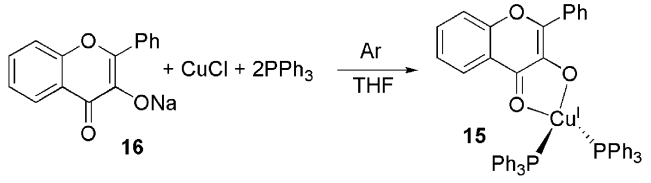
Fig. 2. Probable modes of activation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline and/or dioxygen.

consecutive conversion to **15** and hydrogen evolution (Eq. (4)):



(4)

The product contains approximately 20% **15** depending on the reaction time. However, the presence of **14** in the reaction mixture could be proved by EPR spectroscopy. The solid state spectrum of **14** indicates the presence of an organic radical with a g value of 2.0074 showing fine structure. The solution spectrum of **14** in acetonitrile exhibits hyperfine structure and the coupling constants determined by simulation were found to be: $a_{Cu} = 9.2$, $a_P(2) = 12.0$, $a_H(2) = 1.4$ and $a_H(2) = 0.6$ G. The formation of the electron reduction product **15** from **14** may be important as a radical intermediate in the enzyme reaction since the formation of an endoperoxide by the reaction of a radical species with dioxygen, as proposed in some studies, seems to be a reasonable assumption for the enzyme reaction. The reaction of sodium flavonolate (**16**) with copper(I) chloride in the presence of PPh_3 in THF as solvent leads also to the $Cu^I(fla)(PPh_3)_2$ (**15**) complex in 88% yield (Eq. (5)):

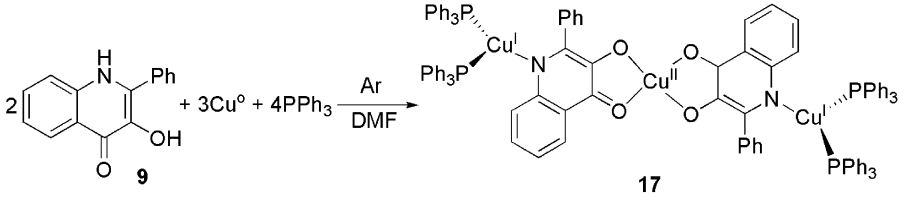


(5)

Compound **15** shows characteristic absorption bands in the visible region at around 430 nm due to the flavonolate ligand [49]. Strong IR bands at 1560 cm^{-1} assigned to ν(CO), showing a decrease of 40 cm^{-1} compared to flavonol [ν(CO) = 1602 cm^{-1}], arise as a result of the formation of a five-membered ring [50]. The complex has a distorted tetrahedral geometry around the copper with the flavonol chelating through the 3-hydroxy and 4-carbonyl groups with Cu–O bond lengths of 2.056(4) and 2.164(5) Å. Due to coordination to the copper ion there is also a change in the bond lengths of the pyranone ring, which may be assigned to delocalization of the π-system over the whole molecule.

2.2. Preparation of copper(I) 1H-2-phenyl-3-hydroxy-4-oxoquinolinate complex [51]

Metallic copper, in the presence of PPh_3, reacts with 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**9**) in DMF under argon to give the trinuclear copper complex $Cu_2^ICu^{II}(Phquin)_2(PPh_3)_4$ (**17**) in 30% yield (Eq. (6)):



(6)

$Cu_2^ICu^{II}(Phquin)_2(PPh_3)_4$ contains a copper(II) ion in a square planar coordination. It is paramagnetic with $\mu_B = 1.64$ and EPR parameters of $g = 2.116$ and $A = 71.1$ G. The other two copper(I) ions coordinate to the deprotonated nitrogen atoms of the heterocycles. It represents a smooth activation of the N–H bond as found in a few cases with other metal complexes too [52]. The result above show that copper metal probably liberates H_2 from **9** to give $Cu^{II}(PhquinH)_2$ (**18**) which in the presence of triphenylphosphine reacts further with scission of the N–H bond to give the trinuclear copper(I)(II) complex **17**. The driving force for these reactions is the formation of the very stable chelate complexes **17** and **18**. **17** cannot be considered as a clear Cu(I) amido complex since the C=N bond length is longer than that in the parent compound and the heterocycle is delocalized [53].

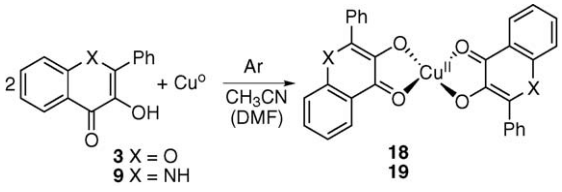
2.3. Preparation of copper(II) flavonolate complex [54,55]

Because the oxidation state of copper in quercetinase is believed to be two, the preparation, characterization and oxygenation studies of copper(II) flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolate complexes are of great interest. Flavonol (**3**) reacts with copper metal in acetonitrile to give (flavonolato)-copper(II) complex (Eq. (7)). The complex $Cu^{II}(fla)_2$ (**18**) was obtained in small quantities, which could be prepared in excellent yield starting from $Cu(OMe)_2$ and flavonol. The complex shows a characteristic absorption band in the visible spectrum at 426 nm due to the flavonolate ligand and an IR absorption band at 1536 cm^{-1} assigned to ν(CO). It is paramagnetic with $\mu_B = 1.76$ and solid state EPR parameters of $g_\parallel = 2.2518$ and $g_\perp = 2.0849$. The complex possesses high symmetry with *trans* coordination of the flavonolate ligands in

Table 1
Comparison of copper(II) flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complexes

	Cu–O (3-ol) (Å)	Cu–O (4-oxo) (Å)	Δ (Cu–O) (Å)
$Cu^{II}(fla)_2$	1.900(2)	1.942(2)	0.042
$Cu^{II}(PhquinH)_2$	1.9067(13)	1.9307(13)	0.024
$Cu_2^{I}Cu^{II}(Phquin)_2(PPh_3)_4$	1.9177(13	1.9286(14)	0.011

a square planar geometry:



(7)

2.4. Preparation of copper(II) 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complex [51]

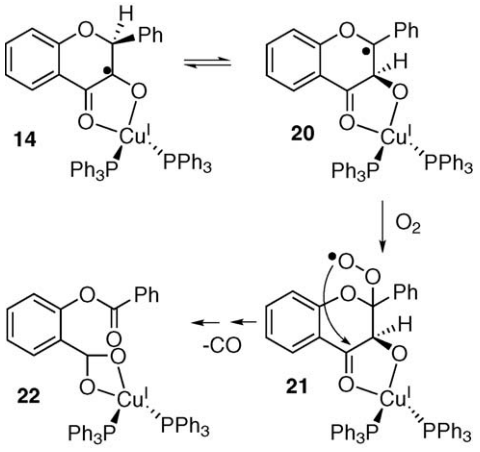
Metallic copper also reacts with 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**9**) in DMF at 50 °C to give the complex $Cu^{II}(PhquinH)_2$ (**19**) in 33% yield. The complex contains a Cu(II) center with $\mu_B = 1.97$ and EPR parameters of $g = 2.128$ and $A = 71.8$ G. Single crystals of $Cu^{II}(PhquinH)_2$ (**19**) suitable for X-ray structure determination were grown from DMF. It shows square planar geometry around the copper(II) ion and the Cu–O distances are somewhat longer and shorter than in the corresponding bis(flavonolato)copper(II) complex (Table 1). The complex has an exactly square planar geometry with *trans* coordination of the oxoquinoline ligand. The Δ|Cu–O| distances of the copper(II) flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complexes show that, in the case of 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complexes the degree of delocalization is higher, resulting in more stable complexes against dioxygen.

3. Biomimetic oxygenation of copper flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complexes

For the elucidation of the possible mechanism of the ring cleavage reaction of the coordinated flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate ligands stoichiometric oxygenations of their copper complexes were carried out.

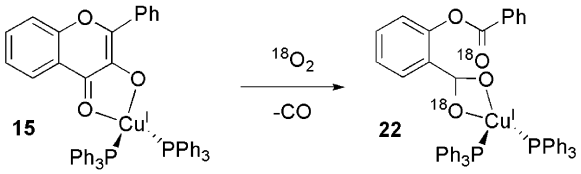
3.1. Oxygenation of copper(I) flavonolate complexes [45,46]

Eq. (5) shows the formation of $Cu^{I}(fla^{\bullet})(PPh_3)_2$ (**14**) by reaction of flavonol with metallic copper in the presence of PPh_3. By carrying out the same reaction under dioxygen a much faster reaction took place. After a short period of time the yellow colored solution turned to intense red which diminished later. We assume that the reason for that is the transient formation of a peroxy radical (**21**) as a result of the reaction of the radical species **14** or **20** with dioxygen. In **21** the peroxy group could attack the 4-carbonyl group of the coordinated flavonolate leading to an endoperoxide which could be presumed to break down to $Cu^{I}(O\text{-bs})(PPh_3)_2$ (**22**) and carbon monoxide (Eq. (8)):



(8)

On recrystallization, the greenish colored product gave a colorless complex **22**, which is diamagnetic with characteristic IR absorption bands of $\nu(CO)$ 1734 cm^{-1} and $\nu(CO_2)$ at 1547 and 1380 cm^{-1}, respectively. The ^{31}P NMR spectrum showed a singlet at −3 ppm proving chemically equivalent phosphines in solution. Crystallographic characterization has shown that $Cu^{I}(O\text{-bs})(PPh_3)_2$ (**22**) is distorted tetrahedral and the carboxylato group of the *O*-benzoylsalicylate ligand is bidentate. $^{18}O_2$ labeling experiments on the oxygenation of $Cu^{I}(fla)(PPh_3)_2$ (**15**) revealed that both ^{18}O-atoms of the $^{18}O_2$ molecule are incorporated into the substrate and the extruded carbon monoxide does not contain ^{18}O, mimicking the enzyme action (Eq. (9)):



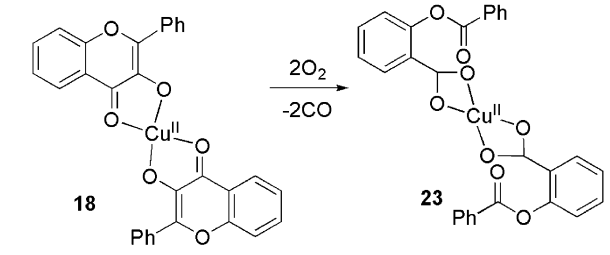
(9)

3.2. Oxygenation of copper(II) flavonolate complexes [46,55]

Oxygenation of $Cu^{II}(fla)_2$ (**18**) in several solvents under ambient conditions (and even faster at temperatures around 100 °C) resulted in the bis(*O*-benzoylsalicylato)copper(II) complex and carbon monoxide contaminated with a small amount of CO_2 (Eq. (10)). Complex **23** has been recrystallized from

either ethanol or pyridine to give $[Cu^{II}(O\text{-bs})_2\cdot EtOH]_2$ (**23b**) and $Cu^{II}(O\text{-bs})_2(py)_3$ (**23a**), respectively. $[Cu^{II}(O\text{-bs})_2\cdot EtOH]_2$ shows an IR absorption at 1740 cm^{-1} [ν(CO)] indicating that the carbonyl group of the benzoyl group is not co-ordinated. The absorptions at 1627 and 1406 cm^{-1} are due to the coordinated carboxylato group. The difference between the asymmetric and symmetric stretching frequencies of the carboxylate group, $\Delta\nu = \nu_{as}(CO_2) - \nu_{sym}(CO_2)$, is 221 cm^{-1} assigning these to a bridging carboxylate bonding mode. In the UV–vis spectrum the 730 nm absorption may be assigned to the d–d transition and the transition at 263 nm to a ligand to metal charge transfer. The room temperature magnetic susceptibility of $\mu_B = 1.30$ is less than expected for two d^9 atoms due to antiferromagnetic interactions as found in many other dicopper carboxylate complexes. For the complex $Cu(O\text{-bs})_2(py)_3$ absorptions due to ethanol are missing and the difference in the asymmetric and symmetric stretching frequencies of the carboxylato group (250 cm^{-1}) is characteristic for monodentate carboxylate coordination. The magnetic moment of $\mu_B = 2.12$ indicates a mononuclear complex as usually found for d^9 ions.

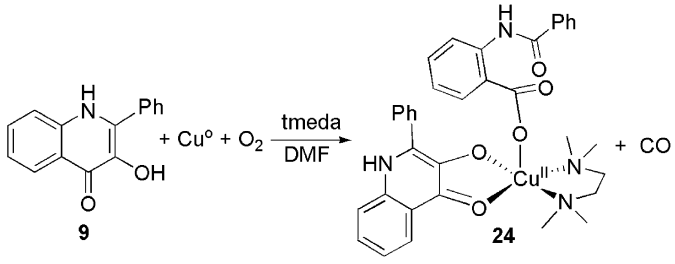
Kinetic measurements were performed in DMF at 90–116 °C in the absence and presence of py. The rate law $-d[Cu^{II}(fla)_2]/dt = k_{obs}[Cu^{II}(fla)_2][O_2]$ describes the kinetic data ($k(M^{-1}\,s^{-1}) = 1.57\times10^{-2}(4.07\times10^{-2}$ (py)), $\Delta H^{\ddagger}(kJ\,mol^{-1}) = 53$, $\Delta S^{\ddagger}(J\,mol^{-1}\,K^{-1}) = -138$). This difference in reaction rate indicates that there are two pathways for the oxygenation of $Cu^{II}(fla)_2$ (**18**), namely a slower process in DMF where the flavonolate ligand is bonded to copper as a stable chelate ligand, and a faster one in the presence of an excess of py which works against the chelate coordination mode making the oxygenation more favored. The reaction fits a Hammett linear free energy relationship with $\rho = -0.63$ for 4′-substituted flavonolates:



(10)

3.3. Oxygenation of copper(II) 1H-2-phenyl-3-hydroxy-4-oxoquinolinate complexes [51]

When during the reaction of metallic copper and 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline in the presence of *N*,*N*,*N*′,*N*′-tetramethylethylenediamine in DMF at 60 °C temperature dioxygen is present even in a very small concentration complex Cu^{II}(*N*-baa)(PhquinH)(tmeda) (**24**) is formed in 41% yield (Eq. (11)):



(11)

During the reaction the C^2–C^3 bond in the 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline is cleaved, both oxygen atoms of O_2 are incorporated into the ligand, and CO is released to give the mixed ligand copper(II) complex Cu^{II}(*N*-baa)(PhquinH)(tmeda). It is paramagnetic with $\mu_B = 1.91$ and EPR parameters of $g = 2.129$ and $A = 55.9$ G. The coordination geometry around the Cu(II) center is slightly distorted square pyramidal with a τ value of 0.06. Addison et al. defined $\tau = 1$ for a regular trigonal bipyramid, while $\tau = 0$ denotes a regular square pyramid [56]. The values of τ between these limits denote the degree of bipyramidicity. The two N-atoms of tmeda and the two O-atoms of the deprotonated 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline occupy basal positions.

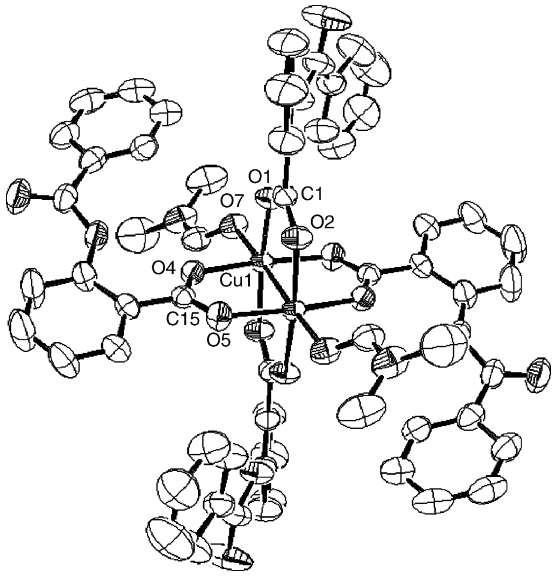
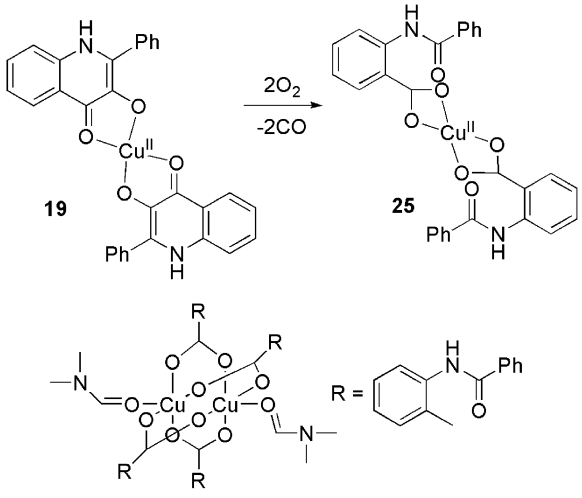


Fig. 3. The molecular structure of $[Cu_2^{II}(DMF)_2(N\text{-baa})_4]$ (**25**) with selected bond distances (Å) and angles (°): Cu(1)–O(1) 1.970(3), Cu(1)–O(4) 1.983(2), Cu(1)–O(7) 2.120(2), Cu(1A)–O(5) 1.948(3), O(1)–C(1) 1.279(4), O(2)–C(1) 1.263(4), Cu(1)–Cu(1) 2.6260(8), O(5)–Cu(1)–O(2) 90.04(12), O(1)–Cu(1)–N(2) 170.78(10), O(2)–Cu(1)–N(1) 167.02(10), N(2)–Cu(1)–N(1) 87.09(11).

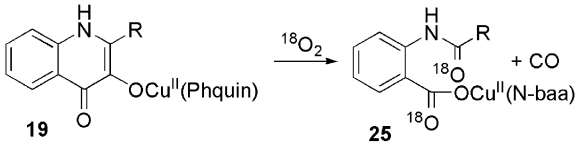
Oxygenation of $Cu^{II}(PhquinH)_2$ (**19**) in several solvents under ambient conditions resulted in the $Cu^{II}(N\text{-baa})_2$ (**25**) complex and carbon monoxide (Eq. (12)). Complex $Cu^{II}(N\text{-baa})_2$ has been recrystallized from DMF by ether diffusion to give blue $Cu_2^{II}(DMF)_2(N\text{-baa})_4$. Single crystals of $Cu_2^{II}(DMF)_2(N\text{-baa})_4$ suitable for X-ray structure determination, crystallized in a monoclinic space group $P21/c$ (Fig. 3). It shows an IR absorption at 1595 cm^{-1} [ν(CO)] indicating that the carbonyl group of the benzoyl group is not coordinated. The absorptions at 1629 and 1461 cm^{-1} are due to the coordinated carboxylato group. The difference between the asymmetric and symmetric stretching frequencies of the carboxylate ligand, $\Delta\nu = \nu_{as}(CO_2) - \nu_{sym}(CO_2)$, is 168 cm^{-1} assigned to a bridging carboxylate bonding mode. In the UV–vis spectrum the d–d transition may be assigned to the 730 nm absorption:



(12)

The room temperature magnetic susceptibility of $\mu_B = 1.35$ is less than expected for two d^9 atoms due to antiferromagnetic interactions as found in many other dicopper carboxylate complexes.

Preliminary kinetic data show first order dependence of the reaction rate on both **19** and O_2. Oxygenations of $Cu^{II}(PhquinH)_2$ were also carried out with a mixture of $^{16}O_2$ and $^{18}O_2$ (40:60) at 60 °C in DMF. After 20 h of stirring the GLC–MS analysis of the gas phase showed only the presence of unlabeled CO. The GLC–MS analysis of the residue, after acidic hydrolysis and treating with ethereal diazomethane, shows the presence of ^{16}O- and ^{18}O-labeled *N*-benzoylanthranilic acid methyl ester in the appropriate ratio. The ^{18}O-labeled *N*-benzoylanthranilic acid methyl ester gave a molecular ion at *m/z* 259 (255 + 4), showing that both ^{18}O atoms of $^{18}O_2$ are incorporated into the carboxylic acid from molecular oxygen (Eq. (13)). The relative abundance of *m/z* 259 to that at *m/z* 255 parallels the $^{18}O_2$ enrichment used in the experiment:



(13)

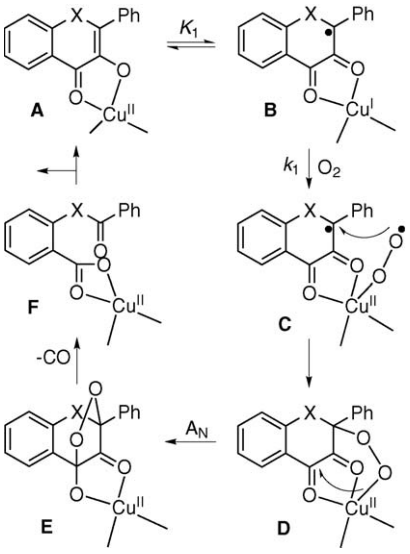
The results outlined above show that copper metal deprotonates 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline to give $Cu^{II}(PhquinH)_2$ which in the presence of triphenylphosphine is further deprotonated through scission of the N–H bond to give the trinuclear copper(I)(II) complex $Cu_2^{I}Cu^{II}(Phquin)_2(PPh_3)_4$. $Cu_2^{I}Cu^{II}(Phquin)_2(PPh_3)_4$ cannot be considered as a clear Cu(I) amido complex since the C=N bond length is longer than that in the parent compound and the heterocycle is delocalized. In the presence of tmeda and dioxygen cleavage of the C^2–C^3 bond in 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline occurs with incorporation of two O-atoms and CO release. Similar results were found on the reaction of $Cu^{II}(PhquinH)_2$ with dioxygen. These reactions resembles the enzyme action on 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline to give the cleavage product as shown earlier.

3.4. Mechanistic pathways in the oxygenation of copper flavonolate and 4-oxoquinolinate complexes

From the experimental data a mechanism for the reaction of the copper flavonolate and 4-oxoquinolinate complexes with dioxygen can be proposed (Eq. (14)). It is known that flavonol (**3**) (and 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**9**) as well) are inert towards dioxygen in their protonated forms. Deprotonation of flavonol and 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline enhances the activity of the corresponding anions toward dioxygen, yielding cleavage products such as those found in the enzyme reactions. Copper(II) has been found in the resting state of quercetinase. Copper(II) ion differs from copper(I) ion in its reactivity toward dioxygen. The latter has a rich dioxygen chemistry while the former is very stable and non-reactive. Now in the case of copper flavonolate and 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate complexes the question arises as to whether simple deprotonation (formation of the copper complex) is the activation step producing free

flavonolate or 1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate ions through dissociation of the complex, or a certain role may also be accorded to copper in the activation process.

On the basis of our results above we suggest a mechanism as shown in Eq. (14). We believe that in a fast pre-equilibrium the copper(II) flavonolate (1*H*-2-phenyl-3-hydroxy-4-oxoquinolinate) complex (**A**) undergoes intramolecular electron transfer from the ligand fla^- ($PhquinH^-$) to Cu^{II} resulting in the copper(I) flavonoxy (1*H*-2-phenyl-3-hydroxy-4-oxoquinoline radical) radical complex **B**. This type of intramolecular electron transfer, called valence tautomerism, can occur when the energy levels of the HOMO orbital of the ligand (substrate) and the LUMO orbital of the metal ion are close to each other. In that case it is possible to shift the equilibrium to either side by changing the temperature as has been shown by EPR spectroscopy in the case of the copper(II) catecholate versus copper(I) semiquinone system [57,58]. The equilibrium is largely shifted to the left (K_1 is rather small). In **B** there are two redox-active centers, the radical ligand and the copper(I) species. The biradical dioxygen may react at both sites, in an oxidative addition to the copper leading to a (superoxo)copper complex **C**, or in a radical–radical reaction with the flavonoxy (1*H*-2-phenyl-3-oxy-4-oxoquinoline radical) ligand. We believe the former is the rate-determining step, supported by the kinetic data and the negative entropy of activation. This is followed then in a fast consecutive formation of the trioxametallocycle **D** which reacts by a nucleophilic addition on the C4 carbon to give the endoperoxide **E**. The reaction rate in DMF was found to be rather slow, which can be explained by the formation of a stable chelate ring which makes the oxygenation unfavored. By using py as coligand the reaction becomes faster, suggesting that py works against the chelate coordination making oxygenation much more favored. On the basis of these results we can argue that the key to the reactivity either in the enzyme or its model systems is the monodentate coordination mode of the substrate molecules. Enzyme studies agree with this assumption. The ES complex also showed that the 4-carbonyl oxygen of the flavonol molecule cannot approach the metal being blocked at a non-coordinating distance of about 3.5 Å [43]:



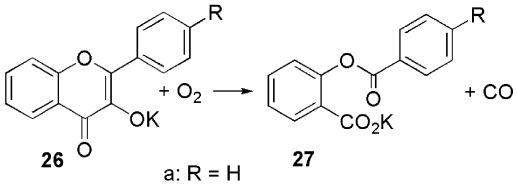
(14)

4. Nonredox metal assisted oxygenation of flavonol and 4-oxoquinoline derivatives

Due to our interest in CO-releasing enzyme models, studies on the base-catalyzed oxygenation of flavonol (**3**) and the isoelectronic 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**9**) were carried out in order to expand our understanding of the biological activities of these compounds and disclose the possible pathway(s) of the enzyme reaction.

4.1. Oxygenation of potassium flavonolate in aprotic sovent [59]

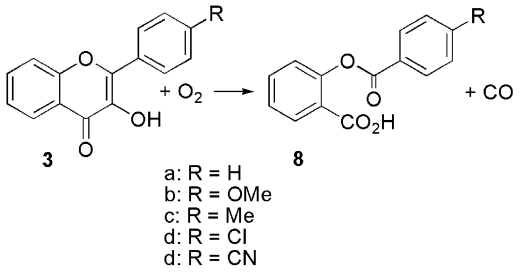
The potassium salts of flavonol and 4′-substituted flavonols were prepared in THF by reacting metallic potassium with flavonols. The salts were characterized by elemental analysis, IR spectra, and UV–vis spectroscopy. The oxygenation of the potassium salts of flavonols (**26**) in DMF at 40 °C leads to the corresponding potassium *O*-benzoylsalicylates (**27**), the enzyme-like products and carbon monoxide (Eq. (15)). Kinetic investigation of the reaction ($-d[flaK]/dt = k[flaK][O_2]$, $k(M^{-1}\,s^{-1}) = 0.328$, $\Delta H^{\ddagger}(kJ\,mol^{-1}) = 29$, $\Delta S^{\ddagger}(J\,mol^{-1}\,K^{-1}) = -161$), has shown that the reaction has a SET mechanism, where electron transfer from the flavonolate ion to dioxygen takes place as the initial step yielding flavonoxy radical and superoxide ion. The presence of the flavonoxy radical could be confirmed by EPR spectroscopy ($g = 2.0038$, $dH = 1.8$ G, $a_H = 0.9$ G). The influence of the 4′-substituted groups on the reaction rate of the oxygenation showed a linear Hammett plot with a reaction constant of $\rho = -0.73$, indicating that electron-releasing groups result in enhanced reactions rates. The linear correlation between the anodic wave of the CV spectra and the reaction rates of the 4′-substituted flavonolates are a good proof for this mechanism:



(15)

4.2. The base-catalyzed oxygenation of flavonol and its 4′-derivatives in protic solvent [60]

The kinetics of the oxygenation of flavonol have been investigated also in a protic solvent, which mimics the circumstances of biological events. Oxygenation of flavonols in 50% DMSO–H_2O resulted in the oxidative cleavage of the heterocyclic ring to give the corresponding depsides and carbon monoxide (Eq. (16)):



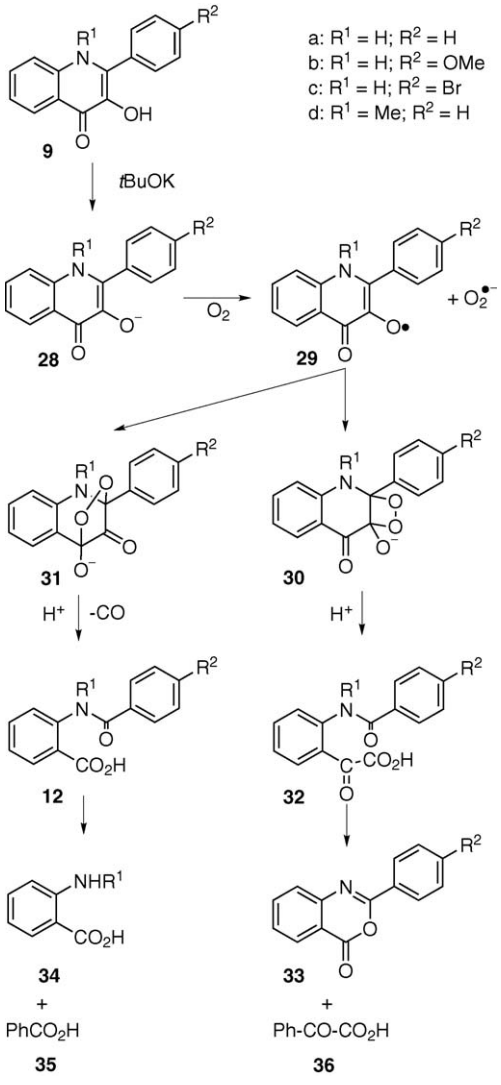
(16)

Kinetic measurements were performed in 50% DMSO–H_2O at 70–90 °C, pH 6.4–10.8 and $I = 0.1\ mol\ L^{-1}$. The rate law $-d[flaH]/dt = k_{obs}[OH^-][flaH][O_2]$ ($k_{obs} = kK/[H_2O]$) describes the kinetic data ($k(M^{-1}\ s^{-1}) = 4.53 \times 10^{-2}$, $\Delta H^{\ddagger}(kJ\ mol^{-1}) = 59$, $\Delta S^{\ddagger}(J\ mol^{-1}\ K^{-1}) = -110$). The reaction showed specific base catalysis and fits a Hammett linear free energy relationship for 4′-substituted flavonols ($\rho = -0.50$). The linear correlation between the oxidation potential of the flavonols and the rate constants is consistent with a higher degree of electron density on the flavonolate ion making them more nucleophilic and the electrophilic attack of O_2 easier.

4.3. The base-catalyzed oxygenation of quinoline derivatives [61]

Oxygenation of 4-oxoquinoline derivatives (**9**) was carried out with O_2 in various solvents such as DMF, DMSO, THF, and MeCN in the presence of potassium *tert*-butoxide. The reaction leads to cleavage products derived from either an endoperoxide (**31**) or a 1,2-dioxetan (**30**) intermediate (Eq. (17)).

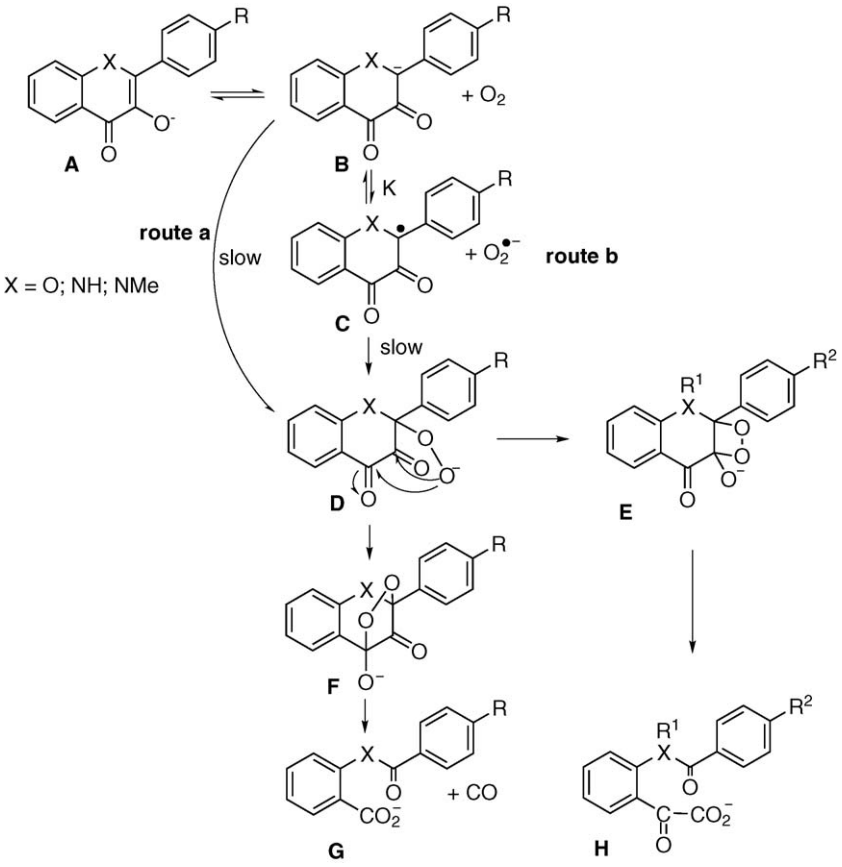
The ratio of the two reaction pathways, based and calculated from the product composition, is dependent on the solvent. Estimated endoperoxide/1,2-dioxetan ratios are as follows: 50:50 (THF), 70:30 (MeCN), 92:8 (DMF) and 50:50 (DMSO). From the results it can be concluded that the base-catalyzed oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**9**) and its 4-substituted derivatives proceeds via a SET mechanism, where the deprotonated anions **28** transfer one electron to dioxygen forming the radicals **29** and superoxide ion. The persistent 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline radical **29a** could be detected by EPR with hyperfine structure ($g = 2.0052$, $a_N = 1.69$, $a_H = 1.19$, $a_{H'} = 1.19$, and $a_{H''} = 0.24$ G):



(17)

4.4. Mechanistic pathways in the oxygenation of flavonols and 4-oxoquinolines

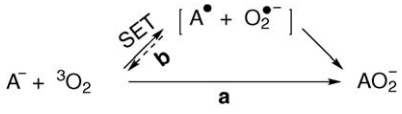
On the basis of the chemical, spectroscopic, inhibition, spin trapping, and kinetic data it appears that the oxygenation of flavonols and quinolines proceeds principally via two mechanistic pathways, a concerted reaction with $^3O_2$ (Eq. (19), route **a**) and/or a stepwise (SET) pathway (Eq. (18), route **b**). The spin restriction can be overcome in both pathways either by a spin change (route **b**) or by the high energy content of the species **A**:



(18)

According to route **a**, the mesomeric form **B** of the deprotonated ligand **A** reacts with dioxygen in an electrophilic, slow rate-determining process to the deprotonated hydroperoxide species **D**. In route **b** the deprotonated ligand **B** undergoes a fast and reversible electron transfer with dioxygen yielding the radical **C** and superoxide ion. This is then followed by the rate-determining step between the two radicals formed (**C** and $O_2^{\bullet -}$) ending up in the peroxidic species **D**. The intramolecular nucleophilic attack of the –O–O^- group on the 4C=O leads to an unstable endoperoxide **F**, which decomposes by loss of CO to *O*-benzoylsalicylic acid (**G**) in fast consecutive steps. In the case of quinolines the intramolecular A_N reaction of **D** on 3C=O leads to the unstable 1,2-dioxetan intermediates **E** too, and their decomposition results then in the phenylglyoxylic acid derivatives **H**.

The nature of the energy barrier in this carbanion–dioxygen reaction is an intriguing mechanistic question. Interaction of an anion with $^3O_2$ requires a change in multiplicity [62]. It was suggested that it occurs after electron transfer from the anion to O_2 ($O_2^{\bullet -}$ is observed as a product whenever electron transfer is exothermic) since a change of electron spin occurs readily in $O_2^{\bullet -}$ (Eq. (19b)). Carbanions of radicals with electron affinities = 20 kcal react with ground state dioxygen while those of higher electron affinities do not, presumably because reaction (19b) has become unfavorable [63]. The reaction of carbanions with $^1O_2$ where no spin change is required (e-transfer is 22 kcal/mol more favorable) is allowed to proceed for carbanions. These facts suggest that in these reactions, basically two pathways are possible (Eq. (19), paths **a** and **b**):



(19)

The first is a SET reaction where electron transfer from the anion A^- to $^3O_2$ is dominant, which is followed by a fast coupling reaction to AO_2^-. Both steps may be rate-determining. If the coupling reaction is the rate-controlling step then the e-transfer must be reversible. The other possibility is the direct electrophilic attack of $^3O_2$ on the anion leading directly to AO_2^-. Whether the carbanion reacts with $^3O_2$ at all depends on the energy level of the HOMO (or redox potential) of A^- as stated before. Whether this reaction will proceed via a SET reaction or a single (concerted) step to the adduct AO_2^- depends mainly on the stability of the radical $A^\bullet$ and reaction conditions either supporting the stability of $A^\bullet$ or decreasing it. The flavonoxy radical (**C**, X=O) is fairly unstable. This is supported by difficulties for its preparation, its short lifetime, and poor detection by EPR. Preliminary calculations showing its high energy content are also in agreement with its stability. Oxygenation of the isoelectronic 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**9**, X=NH), where the radical $A^\bullet$ is much more stable due to nitrogen substitution provides, evidence for this assumption.

5. Conclusion and trends

Flavonols (**3**), 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline and 1*H*-3-hydroxy-4-oxoquinaldine (**9**) are isoelectronic, resistant to

the reaction with triplet dioxygen in their protonated form, however they all are easily metabolized by flavonol 2,4-dioxygenase (with copper ion at its active site) and 3-hydroxyquinolin-4-(1*H*)-one 2,4-dioxygenase (without any metallic or other cofactor) by triplet dioxygen with concomittant release of carbon monoxide. Many questions can be raised in this context, e.g. why one enzyme uses a metal ion cofactor and the other not, what is the role of the copper ion, does the enzyme utilize the copper center its redox ability during the enzymatic process or not. The deprotonated substrates are electron-rich anions which should be able to react with triplet dioxygen to lead to oxgenated products.

The following conclusions can be drawn from the model studies carried out: The one electron oxidation of the deprotonated substrates leads to the corresponding radicals from which the flavonoxy radical is of a very transient nature while the others are very stable even in protic solvents. However all the deprotonated substrates are oxygenated to the enzyme-like products with triplet dioxygen. By the use of radical initiators such as TEMPO, galvinoxyl or DPPH, all the substrates undergo oxygenation by $^3O_2$ to the same products and CO release [64,65]. The deprotonated substrates do coordinate to copper(I) or copper(II) via the 3-OH and 4-C=O groups leading to very stable chelate complexes. Their oxygenation leads also to enzyme-like products; however more severe reaction conditions (elevated temperature) are desired. We assume that valence tautomerism is essential for the copper-assisted oxygenation producing a reactive copper(I) center towards dioxygen and also stabilizing the flavonoxy radical through coordination to be able to react with the bound dioxygen species on the copper. Suggestions on the basis of the studies of flavonol 2,4-dioxygenase and also our own work may lead to the conclusion too, that probably a monodentate coordination of the deprotonated substrate to the copper may inherit a much better reactivity towards dioxygen than the chelated one. Further work needs to be carried out to prove this point. Insofar as the absence of a metallic cofactor in the case of the other two substrates is concerned, the better stability of the assumed intermediate radicals may be a plausible explanation.

Acknowledgements

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