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Kinetics and mechanism of the base-catalyzed oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines in DMSO/H_2O

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ABSTRACT

The oxygenation of 4′-substituted 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines ($PhquinH_2$) in a DMSO/H_2O (50/50) solution leads to the cleavage products at the C2–C3 bond in about 75% yield at room temperature. The oxygenation, deduced from the product compositions, has two main pathways, one proceeding via an endoperoxide leading to CO-release, and the other through a 1,2-dioxetane intermediate without CO-loss. The reaction is specific base-catalyzed and the kinetic measurements resulted in the rate law $-d[PhquinH_2]/dt = k_{OH^-}[OH^-][PhquinH_2][O_2]$. The rate constant, activation enthalpy, and entropy at 303.16 K are as follows: $k_{OH^-} = (2.42 \pm 0.03) \times 10^3$ mol^{-2}L^{2}s^{-1}; $\Delta G^{\ddagger}$=73.13±4.02 kJ mol^{-1}; $\Delta H^{\ddagger}$=70.60±4.04 kJ mol^{-1}; $\Delta S^{\ddagger}$=−28±2 J mol^{-1} K^{-1}. The reaction fits a Hammett linear free energy relationship for 4′-substituted substrates, and electron-releasing groups make the oxygenation reaction faster (ρ=−0.258). The EPR spectrum of the reaction mixtures showed the presence of the organic radical 1*H*-2-phenyl-3-oxyl-4-oxoquinoline and superoxide ion due to single electron transfer from the carbanion to dioxygen. The pathway via 1,2-dioxetane could be proved by chemiluminescence measurements.

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1. Introduction

The biological oxygenation of natural substances by oxygenases is an important part of oxidative metabolic processes.[1] These are mainly catalyzed by metalloenzymes containing usually iron or copper ion at their active sites.[2] The redox behavior of these transition metal ions seems to be significant for the catalytic ability, to the activation of the substrates or molecular oxygen.[3] Among the dioxygenases the so-called CO-releasing dioxygenases form an extremely interesting group since during the catalytic process two C–C bonds are cleaved with concomitant formation of carbon monoxide.[4] It is known that mammalian microsomal heme monooxygenase system forms CO and biliverdin IXa from iron protoporphyrin IX (**1**) in the presence of dioxygen and NADPH.[5,6] Furthermore four more enzymes are known which, in an analogous manner, catalyze the oxidative cleavage of two C–C bonds with CO release, namely, the flavonol 2,4-dioxygenase[7,8] produced by *Aspergillus flavus*[9] and *Aspergillus japonicus*,[7,10] the prokaryotic aci-reductone oxidase from *Klebsiella pneumoniae*,[11] the 1*H*-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase,[12] and 1*H*-3-hydroxy-4-oxoquinoline 2,4-dioxygenase,[13] respectively. The substrates (**2**, **4**) of these dioxygenases show common features as shown in Scheme 1, since they possess a double bound between the C2 and C3, a hydroxyl group at C3, and a carbonyl group at C4.

Flavonol and 1*H*-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase cleave the C2–C3 bond in **2**, **2a**, and **5a** with the incorporation of both *O*-atoms of O_2 into the substrates with release of CO (Scheme 2). The bacterial CO-forming dioxygenases that catalyze the *N*-heterocyclic ring scission reactions in the metabolism of quinaldine and 1*H*-4 oxoquinoline are small monomeric proteins, which cleave their substrates with the help of dioxygen. Flavonol 2,4-dioxygenase (quercetinase) from *A. japonicus* was reported to be a homodimer containing one atom of Cu^{II} per monomer unit.[10]

The structure was recently determined by the X-ray measurement of quercetinase isolated from *A. japonicus* and it was proposed that the copper ion activates flavonol for dioxygen attack by formation of a Cu^{II}–substrate complex.[14] The base-catalyzed autoxidation[15–17] and the copper ion assisted oxygenation of

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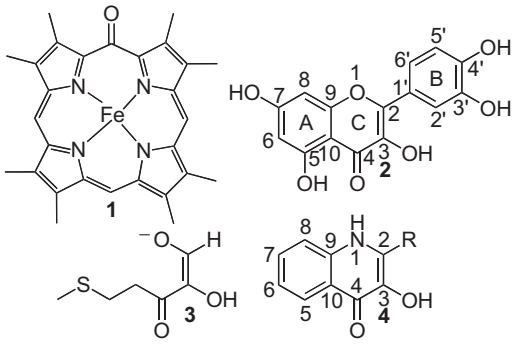
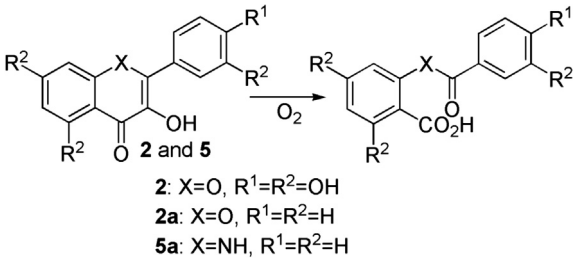


Scheme 1. Substrates for CO-releasing enzymes.



Scheme 2. Flavonol and 1*H*-3-hydroxy-4-oxoquinoline 2,4-dioxygenase action.

flavonols[18] were found to provide a model for the reaction of flavonol 2,4-dioxygenase. In contrast to quercetinase, where Cu^{II} may act as a base or as an electron acceptor due to valence isomerism in copper flavonolates,[19] both 1*H*-3-hydroxy-4-oxoquinoline and 1*H*-3-hydroxy-4-oxoquinaldine 2,4-dioxygenase do not contain any metal or other cofactor.[20] An amino acid residue may act as a base catalyst, however the active site residue(s) proposed to activate the *N*-heterocyclic substrates for O_2 attack remain to be identified. Recent findings on studies of these enzymes and their models with the substrates **2**–**4** suggest that deprotonation leads to the corresponding carbanions first and then direct electron transfer to O_2 resulting in a radical radical pair.[21] This can only happen if the electron affinity of the anions is less than 20 kcal/mol[22] and the formed substrate radical is stabilized.[23] Model reactions revealed that the flavonoxyl radical is less persistent[17] than the one from 2-phenyl-3-hydroxy-4(1*H*)-oxoquinoline being flavonol 2,4-dioxygenase a copper-containing dioxygenase. Suggestions for the formation of the peroxy anion,[24] and also a direct one step ionic mechanism may also be possible for the reaction of carbanions with dioxygen.[25] Recent studies on the base-catalyzed oxygenation of 4′-substituted 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines have shown that there are two main reaction pathways (via endoperoxide or 1,2-dioxetane intermediates) and as a result of stepwise electron transfer from the carbanions to dioxygen radicals are formed in aprotic solvents.[26] In order to elucidate the molecular mechanism of these reactions we studied the kinetics of the base-catalyzed oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines and looked for possible intermediates of the reaction in DMSO/H_2O.

2. Results and discussion

2.1. Oxygenation of 4′-substituted 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines (5a–d)

The oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline and its 4′-bromo-, 4′-methoxy, and 4′-nitro-substituted derivatives in DMSO/H_2O solution at a pH value of 10.4, room temperature, and atmospheric dioxygen pressure for 6 h resulted in the dioxygenolytic cleavage of the *N*-heterocycle (Eq. 1). The O_2-uptake and GC–MS analyses of the products obtained after acidification with HCl and then methylation with diazomethane gave product distribution. Benzoin was used as internal standard. The reaction products showed a diversity of products compiled in Table 1. From the product composition it seems clear that their formation can be deduced from endoperoxide and also from 1,2-dioxetane intermediates. The formation of 1,2-dioxetane could be proved by measurement of the chemiluminescence of the reaction. 1,2-Dioxetanes readily decompose to excited carbonyl compounds, which then emit light and give ground state carbonyls.[27–29] Measuring the emitted light during the reaction in DMSO–water at 20 °C shows that in the first phase of the reaction at optimal concentration there is a maximum of emittance as can be seen in Fig. 1. It may suggest that the 1,2-dioxetane pathway is more dominant in the first part of the reaction and later slowly diminishes. Earlier similar work on flavonols did not give splitting products due to 1,2-dioxetane formation. However, during model studies on the oxygenation of copper(II) flavonolate complexes in one case ketocarboxylatocopper complexes could be isolated and characterized, without CO-loss, which hinted to the formation of 1,2-dioxetane.[30] The data show also that the substituents in the 4′ position influence only slightly the ratio of the two reaction pathways. The solvents have a profound effect on the ratio of the two pathways as has been shown earlier.[26]

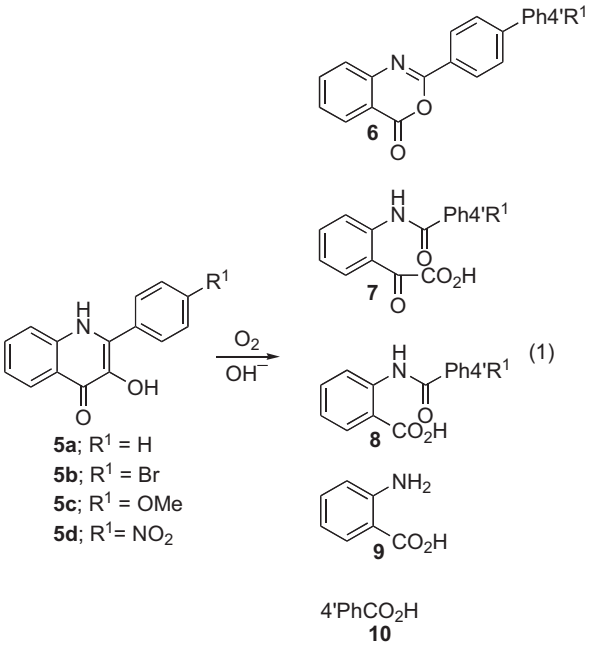


Table 1
Product composition of oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines (**5a**–**d**)

Products/Substrate[a]	4′-H	4′-Br	4′-OMe	4′-NO_2
Benzoic acid	0.2478	0.2015	0.2631	0.2547
Phenylglyoxylic acid	0.1447	0.1526	0.1024	0.3017
Anthranilic acid	0.0914	0.1419	0.2301	0.0804
2-Phenyl-4*H*-3,1-benzox-azin-4-one	0.1501	0.1831	0.1213	0.1422
N-Benzoylanthranilic acid	0.2307	0.2307	0.2026	0.1025
O_2-uptake	0.66	0.73	0.79	0.65

[a] The products formed are given in mmols in relation to 1 mmol substrate.

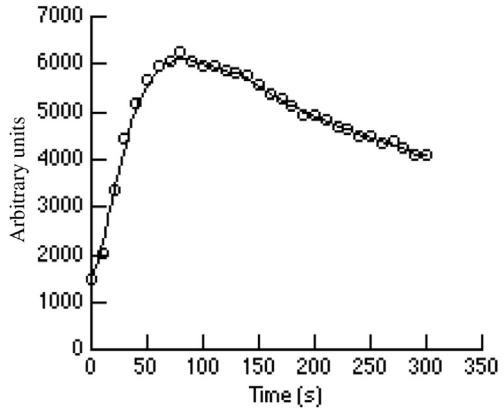


Fig. 1. Time dependence of chemiluminescence of $PhquinH_2$ in alkaline $DMSO/H_2O$ under O_2.

The elimination of carbon monoxide during the reactions could be detected and quantified by GLC–MS and volumetric measurements. The amount of CO formed was also a measure for the endoperoxide pathway at the oxygenation reactions.

2.2. EPR measurements and inhibition experiments

It has been shown earlier that the oxygenation of flavonolates[18] leads to radical species. The base-catalyzed oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines leads also to radical products.[26] In the case of flavonols the flavonoxyl radical could only be detected if the reaction was carried out in aprotic solvent.[31] In $DMSO/H_2O$ mixtures no radical species could be detected by EPR.[32] At the oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines in aprotic solvents, such as DMF, MeCN, THF, and DMSO the presence of persistent 1*H*-2-phenyl-3-oxyl-4-oxoquinoline radicals could be shown.[26] In $DMSO/H_2O$ mixture at various pH values the corresponding 1*H*-2-phenyl-3-oxyl-4-oxoquinoline radical gave a well resolved EPR spectrum with hyperfine structure, as shown in Fig. 2, with the parameters g=2.0036, a_N=1.03, a_H=6.05, $a_{H'}$=1.15, $a_{H''}$=0.9.

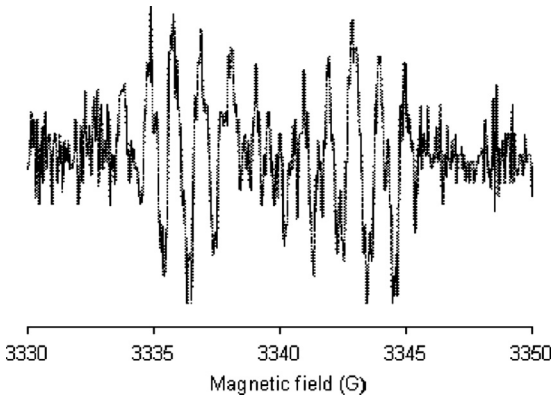


Fig. 2. The EPR spectrum taken during the base-catalyzed oxygenation of $PhquinH_2$ at room temperature in alkaline $DMSO/H_2O$.

However, the concentration of the 1*H*-2-phenyl-3-oxyl-4-oxoquinoline radical formed in solution during the oxygenation process is rather low, and in the UV–vis spectrum no absorption at 532 nm[26] attributable to it could be seen. From that we conclude that the formation of the 1*H*-2-phenyl-3-oxyl-4-oxoquinoline radical during the oxygenation of the deprotonated 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline indicates that a radical reaction pathway occurs in the reaction, but that a non-radical pathway is also possible. In order to check the presence of radicals and to estimate the extent of the radical pathway of the overall reaction inhibition, experiments were carried out by the use of excess 2,6-di-*tert*-butyl-4-methylphenol and ascorbic acid. Kinetic runs with these inhibitors showed no significant decrease in the reaction rate; the rate constant k_{OH^-} at 303.0 K and a pH of 10.6 were found to be 0.50×10^3 and 0.35×10^3 mol^{-2} L^2 s^{-1} (k_{OH^-} = 0.65×10^3 $mol^{-2}L^2s^{-1}$ without inhibitor). The tetrazolium blue test for the superoxide ion was slightly positive.[33] These findings suggest a radical reaction pathway, however, due to the probably greater stability of the 1*H*-2-phenyl-3-oxyl-4-oxoquinoline compared to that of 2,6-di-*tert*-butyl-4-methyl-phenoxyl and ascorbic acid radical no inhibition could be observed. Unfortunately this prevented the determination of the possible ratio of non-radical pathway of the reaction.

2.3. Electrochemistry

The electrochemistry of the 4′-substituted 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines (**5a**–**d**) was carried out under an argon atmosphere and the following conditions: [4′$R^1PhquinH_2$]=7.00×10^{-3} mol^{-1}, [Bu^{t}OK]=1.40×10^{-2} mol L^{-1}, [NBu_4ClO_4]=0.1 mol L^{-1}, scan rate=20 mV/s, with a Pt working electrode in DMF solution at room temperature, NBu_4ClO_4 as supporting electrolyte. The potential values are relative to NHE using an Ag/AgCl reference electrode and corrections taken into account as given in the literature.[34] A typical CV of the deprotonated **5a** can be seen in Fig. 3. The other radicals derived from 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines (**5b**–**d**) resulted in similar spectra (SFigs. 1–3). The corresponding anodic oxidation and cathodic reduction potentials are compiled in Table 2. From the shape of the first redox cycle of the spectra it seems clearly that the one-electron oxidation/reduction of the deprotonated 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline exhibits good reversibility or quasi-reversibility. A comparison with the CV spectra of the *O*-analogs (flavonols)[17,35] show that the radicals formed from the 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines are much more persistent than those formed from flavonolates.

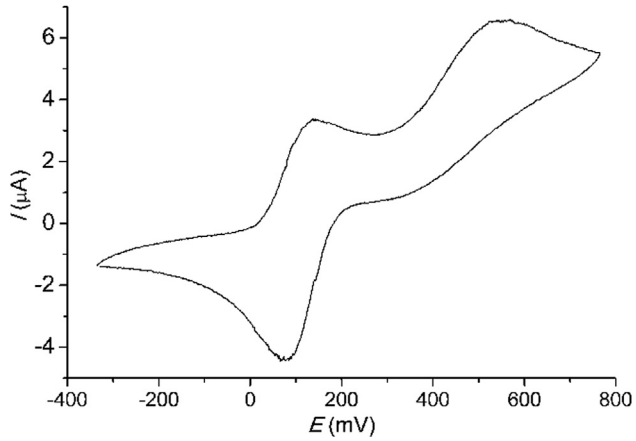


Fig. 3. The cyclic voltammogram of deprotonated 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (**5a**) in DMF under argon atmosphere.

Table 2
Redox potentials of the deprotonated 4′$R^1PhquinH_2$ (**5a**–**d**) in DMF

R^1	E_{ox1} (mV)	E_{red1} (mV)	E_{ox2} (mV)
H	140.8	82.0	556.0
MeO	110.2	41.7	566.0
Br	182.0	89.0	551.0
NO_2	219.0	133.0	—

This is also supported by the good quality of the EPR spectra with hyperfine structures and the persistence of the radicals for days under the conditions.

*2.3.1. Determination of the pK values of 4′R¹PhquinH₂ (**5a–d**).* The determination of the dissociation constants of 4′R¹PhquinH₂ in 50/50% DMSO/H₂O at 25 °C and I=0.2 mol L⁻¹ (KNO_3) was carried out with a Radometer pHM93 instrument and a Metrohm 60222 combination electrode. The electrode was conditioned for 3 days in the same solvent before the measurements were made. The titration was done with a 0.2 mol L⁻¹ KOH solution and its concentration was determined by pH-metry using the Gran relation to be 0.1978 mol L⁻¹. The titrations were carried out with a Metrohm 715 Dosimat automatic burette. The readings of the instrument were converted to concentrations by the Irving method.[36] The autoprotolysis constant of water (p*K*) was found to be 15.412 under the conditions applied. The dissociation constants of the 4′RPhquinH₂ derivatives were determined by pH-metric method at 2 and 1 mmol L⁻¹ analytical concentrations. The results were evaluated by the program SUPERQUAD.[37] The obtained p*K* values are shown in Table 3.

Table 3
The measured p*K* values 4′R¹PhquinH₂ (**5a–d**) in DMSO/H₂O (50/50) at 25 °C and I=0.2 mL⁻¹ (KNO_3)

R¹	p*K*
H	10.43 (1)
MeO	10.63 (1)
Br	10.93 (4)
NO_2	9.47 (1)

2.4. Kinetic measurements

The kinetic measurements on the oxygenation of 4′-substituted 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines (4′R¹PhquinH₂) in DMSO/H₂O were followed by UV–vis spectroscopy under pseudo-first-order conditions. The ionic strength I=0.1 mol L⁻¹ (KNO_3), the pH, the dioxygen concentration, and the temperature were kept constant during the experiments. The rate constants k_{obs} according to Eq. 2 were determined.

$$\text{reaction rate} = k_{obs}\left[4'R^1\text{PhquinH}_2\right] \quad (2)$$

Under these conditions a typical variation of the electron spectrum in the range 290–500 nm as a function of time can be seen in Fig. 4. During the reaction the band at 363 nm decreases while that at 305 nm increases and there is an isosbestic point at 320 nm. The reactions were carried out at higher pH values too. Without added base no oxygenation reaction occurs at all. The time profile of the oxygenation reaction is shown in SFig. 4 and the log [PhquinH₂] versus time diagram (SFig. 5) resulted in a straight line, which is characteristic for first-order dependence in the substrate. This could be also supported by measuring the initial reaction rates of the oxygenation reaction with varying initial substrate concentrations of PhquinH₂. Plots of the reaction rate versus the initial PhquinH₂ concentration resulted also in a straight line (SFig. 6) reinforcing the first-order dependence in PhquinH₂. The rate dependence on the dioxygen concentration was determined by measuring the pseudo-first-order rate constants k_{obs} at different dioxygen concentrations. A straight line of the reaction rate versus dioxygen concentration (SFig. 7) shows that the reaction has a first-order dependence on the dioxygen concentration. The effect of the pH on the reaction was evaluated by carrying out the reactions at various pH under pseudo-first-order conditions. The resulted straight line indicated a first-order dependence (SFig. 8), while carrying out the measurements at one pH value and different buffer concentrations (SFig. 9) showed no difference in the reaction rates.

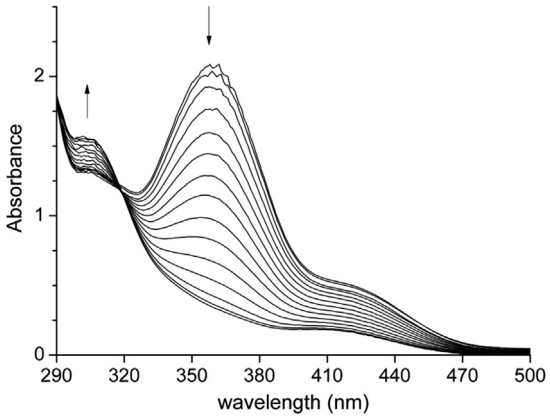


Fig. 4. Change in the electronic absorption spectra of PhQuinH₂ in the presence of base under dioxygen. [PhquinH₂]=2.00×10⁻³ mol L⁻¹, [O₂]=2.69×10⁻³ mol L⁻¹, pH=10.4, [Buffer]=2.39×10⁻² mol L⁻¹, I=0.1 mol L⁻¹, DMSO/H₂O, 20 °C.

This means that the reaction is specific base-catalyzed and the rate expression (3) describes the reaction velocities (STable 1).

$$\text{reaction rate} = k_{OH^-}\left[OH^-\right]\left[4'R^1\text{PhquinH}_2\right][O_2] \quad (3)$$

The rate constant k_{OH^-} at 303.16 K was found to be (2.42±0.03)×10³ mol⁻². The influence of 4′-substituted groups on the reaction rate of the oxygenation showed a linear Hammett plot with a reaction constant of ρ=−0.258 (Fig. 5, STable 2), indicating that electron-releasing groups result in enhanced reaction rates. We also investigated the dependence of the reaction rates on the first anodic oxidation potential of the deprotonated substrates **5a–d**. Fig. 6 shows that substrates with electron-releasing groups have

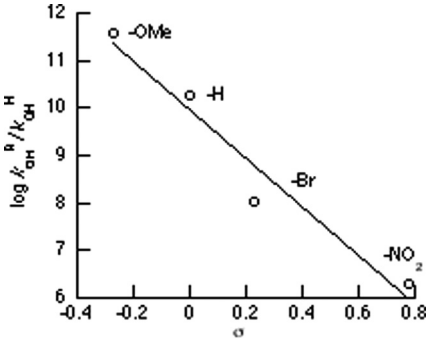


Fig. 5. Hammett plot for the oxygenation of 4′R¹PhquinH₂. [4′R¹quinH₂]=2.00×10⁻³ mol L⁻¹, [O₂]=2.16×10⁻³ mol L⁻¹, pH=10.0, I=0.1 mol L⁻¹, DMSO/H₂O (50/50), 20 °C.

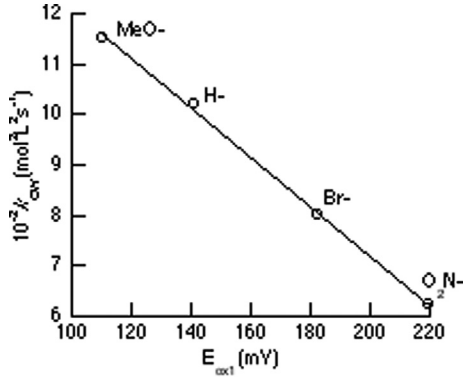


Fig. 6. The dependence of the reaction rate on the first oxidation potentials of the substrates 4′R¹PhquinH₂.

a more negative anodic oxidation potential and also a higher reaction rate. The linear correlation between the anodic oxidation potential and the reaction rate supports that a higher electron density on the deprotonated substrates **5a**–**d** makes the electron transfer easier or enhances the nucleophilic character of the carbanions in their reaction with dioxygen. The kinetic and the EPR data on the oxygenation reactions of the carbanions of the substrates **5a**–**d** show similar features to flavonolates,[17] phenolates,[38] and carbanions.[39]

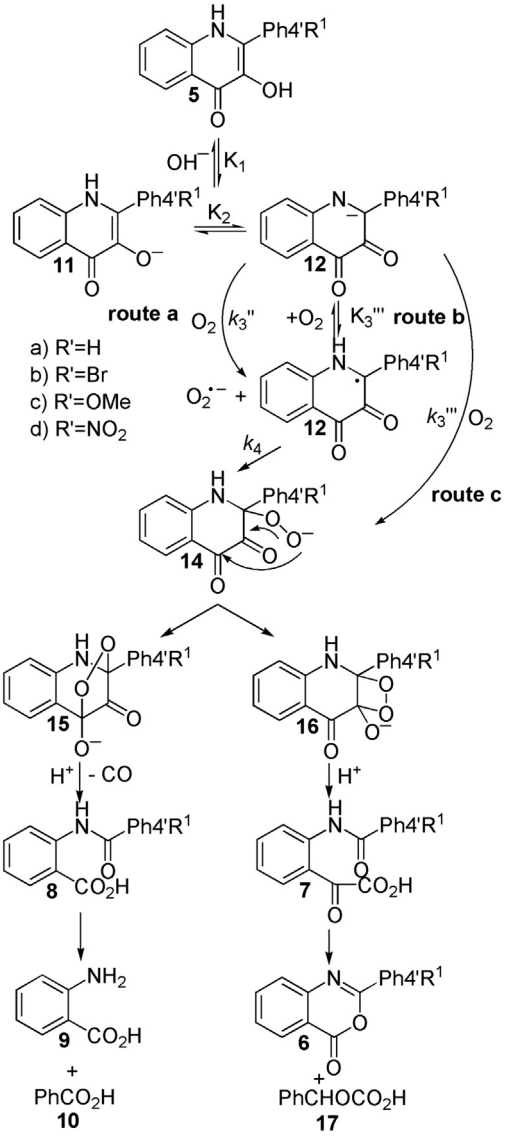
The reduction power of the carbanions formed from **5a**–**d** is high and a direct one electron transfer to dioxygen gives radicals, from which the radicals formed from **5a**–**d** exhibit relative good stability (like the phenoxyl radicals in general),[38] while those formed from carbanions and flavonolates are much less stable. Since the first electron reduction of dioxygen to superoxide ion is unfavorable ($E^{\circ}=-0.62$ V vs NHE in DMF),[40] the reduction potential of phenolates,[41] carbanions,[42] flavonolates,[17] and the carbanions from **5a**–**d** are more negative than that of O_2, and so the electron transfer is facilitated. The stability of the radicals formed is dependent on the delocalization of the unpaired electron. According to that, the stability of the flavonoxy radicals is rather low while the phenoxy and the 4′R^{1}PhquinH• radicals are more stable. The stability depends of course on the solvent used too. While water is considered in many cases as a good quencher for radicals, the PhquinH• radical shows relatively good stability and persistence, and an EPR spectrum with hyperfine structure as shown in Fig. 1 could be recorded. A detailed study of the EPR spectra of the 4′R^{1}PhquinH• radicals in aprotic solvents will be shown in a forthcoming paper.

The third-order rate expression (3) and the non-dependence of the reaction rate on the buffer concentration shows that the reaction is specific base-catalyzed and the reaction rate is dependent on the anions 4′R^{1}PhquinH$^-$ and dioxygen. The possible reaction pathways, which satisfy the kinetic data are summarized in Scheme 3. In the first reversible step, the substrates **5a**–**d** are deprotonated to the *O*-anions **11**, which show valence isomerism to give the carbanions **12**. These steps are fast preequilibria and the concentration of **11** and **12** together can be calculated for various pH values from the corresponding p*K* values of **5a**–**d** determined potentiometrically as listed in Table 3. There are more routes from that point, which satisfy the kinetic data. Route a concerns a possible rate-limiting step as a single electron transfer between 4′R^{1}PhquinH$^-$ and dioxygen leading to 4′R^{1}PhquinH• and superoxide ion. We believe however that this route can be ignored, because this step cannot be rate determining since electron transfers from electron-rich substrates to dioxygen are usually fast reactions.[35] The fast reversible reaction of **12** with dioxygen (route b) can lead to the radicals **13** and superoxide anion, and their reaction in a rate-determining step (k_4) results in the peroxidic compounds **14**. These steps are also evidenced by detecting the radical **13a** by EPR spectroscopy and proving the presence of the superoxide ion by the tetrazolium blue method.[33] However, the concentration of the radicals **13** and also that of superoxide anion were rather low. The direct reaction of singlet carbanion with triplet dioxygen requires a change in multiplicity.[17] It occurs after electron transfer from the anion to O_2 since a change in electron spin occurs readily in $O_2^{\cdot -}$. Carbanions of radicals with electron affinities=20 kcal/mol react with ground state dioxygen while those of higher ones do not because it becomes unfavorable.[24] The peroxidic species **14** can also be formed from **12** by route c, where a direct electrophilic attack of dioxygen on the carbanion **12** is rate-limiting. Whether the carbanion reacts with $^3O_2$ at all depends on the energy level of the HOMO orbital (or redox potential) of the anion 4′R^{1}PhquinH$^-$. Whether this reaction proceeds via a radical pathway or through a single (concerted) step to the adduct 4′R^{1}PhquinHO$_2^-$ depends mainly on the stability of the radicals 4′R^{1}PhquinHO$_2^{\cdot}$ and the reaction conditions either supporting its stability or not. We believe that the reaction proceeds by route c and/or by the SET reaction as indicated in route b, while route a can be dismissed. The quantitative measure on the concentration of the radical **13** by UV–vis spectroscopy showed only a small concentration during the reaction. While this does not account for the less favored pathway b, the relative stability of the radical **13** and the slow reaction between **13** and the superoxide anion hints to a smaller extent of this route. According to Scheme 3 the rate Eqs. 4 and 5 can be deduced, where the k_{OH^-} may be expressed as shown in Eq. 6.

$$-\mathrm{d}\left[4'\mathrm{R}^1\mathrm{PhquinH_2}\right]/\mathrm{d}t = \frac{k_4K_1K_2K_3''}{[\mathrm{H_2O}]}\left[\mathrm{OH^-}\right]\left[\mathrm{R_1PhquinH_2}\right]\left[\mathrm{O_2}\right] \tag{4}$$

$$-\mathrm{d}\left[4'\mathrm{R}^1\mathrm{PhquinH_2}\right]/\mathrm{d}t = \frac{k_3'''K_1K_2}{[\mathrm{H_2O}]}\left[\mathrm{OH^-}\right]\left[4'\mathrm{R}^1\mathrm{PhquinH_2}\right]\left[\mathrm{O_2}\right] \tag{5}$$

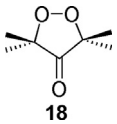
$$k_{\mathrm{OH}} = \frac{k_4K_1K_2K_3''}{[\mathrm{H_2O}]} = \frac{k_3'''K_1K_2}{[\mathrm{H_2O}]} \tag{6}$$



Scheme 3. The possible mechanisms of the base-catalyzed oxidation of 4′R^{1}PhquinH$_2$.

The consecutive fast reaction steps of the peroxidic species **14** may follow two alternatives. In the one, due to intramolecular nucleophilic attack on the C4 carbon, the endoperoxides **15** are formed, which after simultaneous cleavage of the O–O bond and concomitant elimination of CO result in the compounds **8**, **9**, and benzoic acid.

That is the enzymatic pathway. The peroxidic species **14** may also undergo an intramolecular nucleophilic attack on the C3 carbon, leading to a 1,2-dioxetane species, which after cleavage of the O–O bond gives the compounds **7**, **6**, and phenylglyoxalic acid without loss of CO. This represents a non-enzymatic pathway. All attempts to isolate the endoperoxide **15** and **16** failed suggesting that this type of endoperoxides is very unstable if compared with other endoperoxides where CO elimination is not feasible.[43,44] Until now no endoperoxides of this general structure (**18**) could be isolated.



18

1,2-Dioxetanes have been isolated and characterized, however only with bulky groups on the carbon atoms.[45,46] Only indirect evidence for its presence could be found, e.g., by the photochemical oxidation of quercetin and its derivatives[47,48] where only the cleavage products could be isolated. From the temperature dependence of the reaction rates (SFig. 10) the activation parameters at 303.16 K as $\Delta G^{\ddagger}$=73.13±4.02 kJ mol^{-1}; $\Delta H^{\ddagger}$=70.60±4.04 kJ mol^{-1}; $\Delta S^{\ddagger}$=−28±2 J mol^{-1} K^{-1}, and E_A=73.23 ±4.02 kJ mol^{-1}could be calculated. These data also support, the negative entropy of activation, a bimolecular step as the rate-determining reaction in routes b or c.

3. Conclusion

From the results obtained the conclusion may be drawn that the oxygenolysis of the 4′-substituted 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines is a specific base-catalyzed reaction and the kinetic data revealed a first-order dependence with respect to the 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines and dioxygen. The p*K* values of the 1*H*-2-phenyl-3-hydroxy-4-oxoquinolines were determined by potentiometric titrations. The product distribution of the oxygenation reactions indicates endoperoxides and 1,2-dioxetanes as intermediates. For the reaction mechanism two routes are proposed. One (route b) involves an SET reaction from the carbanions **12** to the dioxygen proceeding in a fast reversible reaction yielding the 1*H*-2-phenyl-3-oxyl-4-oxoquinoline radicals **13** and superoxide anion, which react in the rate-determining step to the peroxidic species **9**. These than undergo an intramolecular nucleophilic attack either on the C3 or C4 to 1,2-dioxetanes or endoperoxides, which break down to the products in fast reaction steps. The other route may involve a direct electrophilic attack of O_2 on the carbanions **12** in the rate-determining step leading to 4′R^{1}PhquinHO$_2^-$ (**9**), which are followed by fast consecutive reactions to the products. Route b has been evidenced by the presence of the radical PhquinH• detected by EPR. Route a can be excluded due to data found in the literature for fast electron-transfer reactions from carbanions to dioxygen, while route c is possible and seemed to be the dominant pathway in the case of flavonols in protic solvents. The better stability of the radicals 4′R^{1}PhquinH• may render these reactions dualistic pathways.

4. Experimental section

4.1. General: materials and methods

All manipulations were carried out under pure argon or dinitrogen atmospheres unless otherwise stated using standard Schlenck-type inert gas techniques.[48] Solvents used for the reactions were purified by literature methods[49] and stored under argon. 1*H*-2-Phenyl-3-hydroxy-4-oxoquinoline, 4′-bromo-, 4′-methoxy-, and 4′-nitro-1*H*-2-phenyl-3-hydroxy-4-oxoquinolines were prepared according to literature methods.[50] Diazomethane was freshly prepared by a known procedure in ether and immediately used for the methylation reactions.[51] For the preparation of the buffer for pH=9.6 2.22 g KNO_3 was dissolved in a mixture of 50 mL 0.1 mol L^{-1} sodium hydrogen carbonate and 5 mL 0.2 mol L^{-1} sodium hydroxide then the solution was diluted to 100 mL.[52] The UV–vis spectra were recorded on a Shimadzu UV-160A and Agilent 8453 diode-array UV–vis spectrophotometer and the IR spectra on an Avatar 330 FT-IR Thermo Nicolet instrument and a Specord M80 (Carl Zeiss, Jena) infrared spectrophotometer. The EPR spectra were taken on a JEOL JES- FE/3X EPR spectrometer of X band (9.64 GHz, 200 μW) at room temperature and in DMSO+water mixture. The product analyses were carried out on a Hewlett Packard 4890D gas chromatograph. The mass spectra were recorded on a Hewlett Packard 5890 II, 5971 GC/MSD (75 eV) mass spectrometer. The chemiluminescent measurements were carried out on a TECAN Infinite M200 instrument, microplate 96 well: in each well samples added with automatic injection. The dioxygen concentration was determined by the use of a Beckman Fieldlab Oxygen Analyzer. The sensor was calibrated with dioxygen saturated DMF.[32] For the potentiometric titrations a Metrohm 715 Dosimat automatic burette was used. Cyclic voltammograms were taken on a VoltaLab 10 potentiostat with Voltamaster 4 software for data processing. A platinum working electrode, auxiliary electrode, and Ag/AgCl with 3 M KCl reference have been used. The potentials were referenced versus the ferrocene/ferrocenium (Fc/Fc^+) redox couple. The CV spectra were measured in argon-saturated DMF using 0.1 M tetrabutylammonium perchlorate (TBAP) as electrolyte. Elemental analyses were carried out at the University's analytical unit.

4.2. The bulk oxygenation of 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (PhquinH$_2$)

PhquinH$_2$ (237.25 mg, 1 mmol) in a DMSO/H_2O mixture (10 mL), at 25 °C, 1 bar O_2 pressure were oxygenated for 6 h in a thermostated reaction vessel attached to a buret. The evolved CO was determined by GC–MS from the gas phase and was found to be 0.86 mmol (86%). The reaction mixture was acidified and treated with diazomethane and then analyzed by GC. Gas chromatogram and the conditions with the results are shown in SFig. 11.

4.3. Kinetic measurements

The reaction of 1*H*-2-phenyl-3-hydroxy-4-oxoquinoline (PhquinH$_2$) with O_2 was performed in DMSO/H_2O (50/50) solutions. In a typical experiment PhquinH$_2$ was dissolved in DMSO under an argon atmosphere in a thermostated reaction vessel and a buffer solution was added to adjust the pH value. The reaction vessel was equipped with an inlet for sampling with a syringe and connected to a mercury manometer to maintain constant pressure. The solution was then heated to the appropriate temperature. A sample was taken by syringe and the initial concentration of PhquinH$_2$ was determined by UV–vis spectroscopy measuring the absorbance of the reaction mixture at 363 nm (λ_{max}=363 nm, ε=4885 mol^{-1} L cm^{-1} of PhquinH$_2$). The argon was then replaced by dioxygen and the consumption of PhquinH$_2$ was analyzed periodically (ca. every 5 min).

The rate of consumption of $PhquinH_2$ was independent of the stirring rate, excluding eventual diffusion control effects. Experimental conditions are summarized in STable 1. The temperature was determined with an accuracy of ±0.5 °C; the concentrations of $PhquinH_2$ were measured with a relative error of ca. ±2%; the pressure of the dioxygen was determined with an accuracy of ±0.5%.

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Supplementary data

Kinetic data and graphs, CV spectra, chemiluminescence measurements, and product identification are described. Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.tet.2013.05.117.

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