Kinetics and Mechanism of the Base-Catalyzed Oxygenation of Flavonol in DMSO−H_2O Solution

Éva Balogh-Hergovich[†] and Gábor Speier*[,†,‡]

Research Group for Petrochemistry, Hungarian Academy of Sciences, 8201 Veszprém, Hungary, and Department of Organic Chemistry, University of Veszprém, 8201 Veszprém, Hungary

speier@almos.vein.hu

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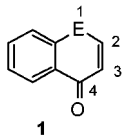
The kinetics of the base-catalyzed oxygenation of flavonol have been investigated in 50% DMSO−H_2O solution in the pH range 6.4−10.8 and an ionic strength of 0.1 mol L^{-1} using spectrophotometric techniques at temperatures between 70 and 90 °C. The rate law $-d[flaH]/dt = k_{obs}[OH^-][flaH][O_2]$ ($k_{obs} = kK_1/[H_2O]$) describes the kinetic data. The rate constant, activation enthalpy, and entropy at 353.16 K are as follows: k/mol^{-1} L s^{-1} = (4.53 ± 0.07) × 10^{-2}, $\Delta H^{\ddagger}$/kJ mol^{-1}= 59 ± 4, $\Delta S^{\ddagger}$/J mol^{-1} K^{-1} = −110 ± 11. The reaction showed specific base catalysis. It fits a Hammett linear free energy relationship for 4′-substituted flavonols and electron-releasing substituents enhanced the reaction rate. The linear correlation between the oxidation potential of the flavonols and the rate constants supports that a higher electron density on the flavonolate ion makes them more nucleophilic and the electrophilic attack of O_2 easier.

Introduction

N- and O-heterocyclic compounds are broadly spread in nature.[1−4] Among them there are a large number of benzopyrone derivatives (**1a**, e.g., flavonoids; quercetin) in plants and the isoelectronic N-compounds, the quinolones (**1b**) are also found in coal tar and crude oil, etc. From the benzopyrone derivatives the flavonoids (**2**) and from the quinolones the 3-hydroxy-2-alkyl derivatives of **1b** desire attention. The formers exhibit a large array of biological activities such as antiinflammatory[5]and anticarcinogenic[6] behavior. This may be due to their ability



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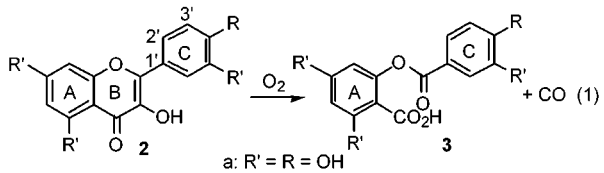
a: E = O
b: E = NH

to bind metal ions and/or undergo oxidation by O_2. This may involve either the catecholate moiety of **2a** (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



a: R' = R = OH
b: R' = R = H
c: R' = H, R = OMe
d: R' = H, R = Me
e: R' = H, R = Cl
f: R' = H, R = CN

role of both classes of compounds on the one side and the mechanistic aspects of oxidative degradation on the other. Studies of this type may contribute to our understanding of enzyme actions involved in the processes. Quercetin (**2a**) and flavonols (**2b**−**f**) are cleaved at the B ring by quercetin 2,3-dioxygenase (a copper-containing enzyme) (eq 1).[7−18] In a similar manner, in 1*H*-3-hydroxy-4-oxoquinoline (**4a**) and 1*H*-3-hydroxy-4-oxoquinaldine (**4b**) the B ring is cleaved by a dioxygenase in a similar way with concomitant CO extrusion, which does not contain metal ion.[19,20] We do not know at present the

* To whom correspondence should be addressed. Tel: +36 88 422 022/4657. Fax: +36 88 427 492.
† Research Group for Petrochemistry.
‡ University of Veszprém.

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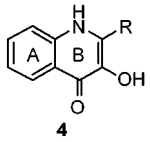

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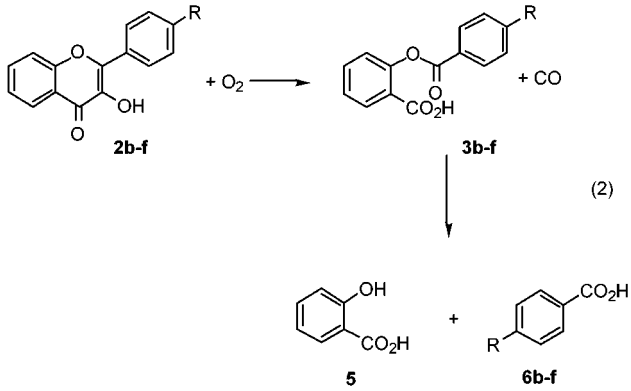
a: R = H
b: R = CH_3
c: R = Ph

reason why in one case a metal ion is needed and in the other not. Model studies on quercetin dioxygenase have already been carried out. Metal complexes of copper[21−32] and cobalt[33−36] have been found to act as catalysts for the oxygenation reaction. Photosensitized oxygenation of flavonols has been studied as a nonenzymic model.[37−39] Base-catalyzed oxygenation of quercetin and flavonols under aqueous[40,41] and nonaqueous[42] conditions has resulted in enzyme-like products as well. Base-catalyzed oxygenation of flavonols (**2b**−**f**) and 2-alkyl-3-hydroxyquinolines in protic solvents may expand our understanding of the biological activities of these compounds and give answers to the questions mentioned previously. Kinetic investigation of the oxygenation of potassium flavonolate in aprotic solvent has shown that the reaction has a SET mechanism, where electron transfer from the flavonolate ion to O_2 takes place as the initial step, yielding flavonoxy radical and superoxide ion.[43] As a continuation of this work, it was of interest to us to extend investigations on the kinetics of the oxygenation of flavonol in protic solvent, since until now, no kinetic studies were carried out under such conditions. This would mimic the circumstances of biological events. Here, we report kinetic studies of the oxygenation of flavonol in 50% DMSO−H_2O solution at constant ionic strength and in the pH range from 6.4 to 10.8.

Results

Oxygenation of Flavonol. Oxygenation of 4′-substituted flavonols (**2b**−**f**) in 50% DMSO−H_2O resulted in the oxidative cleavage of the heterocyclic ring to give the corresponding depsides (**3b**−**f**) and carbon monoxide. Bulk oxygenation of flavonol (**2b**) led to the formation of *O*-benzoylsalicylic acid (**3b**) (∼2%) and carbon monoxide and the hydrolyzed products salicylic acid (**5b**, 83%) and benzoic acid (**6b**, 82%) (eq 2).



2b-f **3b-f** **5** **6b-f** (2)

Kinetic Measurements. The kinetic measurements of the oxygenation of flavonol in 50% DMSO−H_2O were performed at 80 °C, pH = 6.4−10.8 and I = 0.1 mol L^{-1} under pseudo-first-order conditions.[44] The reactions were followed by UV−vis spectroscopy at 462.5 nm, a typical band of the flavonolate ion. No band could be seen at 344 nm assigned to flavonol, indicating that at pH = 10 all of the flavonol is in deprotonated form. From the change of the UV−vis spectrum of flavonol in its reaction with dioxygen (Figure 1, Supporting Information) and the concentration time profile (Figure 2, Supporting Information), the plots of log [flaH] against time showed good linearity (R = 99.95%), indicating that the reaction order with respect to the flavonol is one. This was also confirmed by the straight line obtained by plotting the initial reaction rate against the initial flaH concentration (Table 1, Figure 3, Supporting Information). The first-order dependence of the reaction rate on the dioxygen concentration was established by plotting the initial reaction rate against the O_2 concentration at two substrat to dioxygen ranges (Figure 4, Supporting Information). On the basis of the kinetic data, the rate eq 3 could be derived.

$$\text{reaction rate} = k\,[\text{fla}^-][\text{O}_2] \qquad (3)$$

In basic media, flavonol (flaH) is deprotonated in a fast preequilibrium to yield the flavonolate anion (fla^-), which reacts with dioxygen in the rate-determining step. At pH = 10, however, the deprotonation is complete; therefore, the concentration of the initial flavonolate concentration $[fla^-]_o$ is identical with the initial flavonol concentration $[flaH]_o$.

The reaction rate showed a pH dependence as shown in Figure 1 (Table 2, Supporting Information). At low

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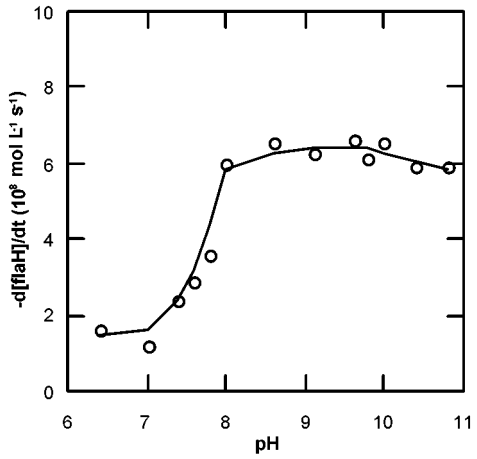
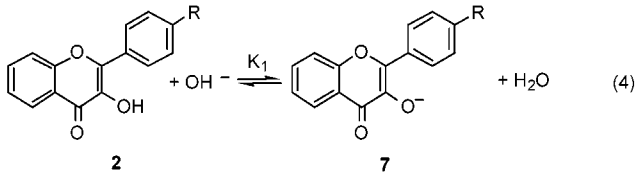


Figure 1. Hydrogen ion dependence of reaction rate. Reaction conditions: 50 mL DMSO/H_2O (1:1 v/v), 80 °C, [flaH$_o$] = 8.0 × 10^{-4} mol L^{-1}, I = 0.1 mol L^{-1}, [O_2] = 1.94 × 10^{-3} mol L^{-1}.

hydroxide ion concentrations (pH < 8), slower reaction rates were measured. In the pH range between 7 and 8 in addition to the absorption band at 462.5 nm (flavonolate) another band at 344 nm (flavonol) can be seen in the UV−vis spectrum, indicating that the flavonol is only partially deprotonated. From the UV−vis spectrum the value of K_1 (2.88×10^7) for the equilibrium (4) could be calculated by using eq 5. No band can be seen at 462.5



(4)

nm below pH 7, excluding the presence of flavonolate ions in the reaction mixture. Runs were also performed at constant pH and different buffer concentrations, where the reaction rate showed no dependence on buffer concentrations supporting specific base catalysis (Table 3, Figure 5, Supporting Information).

$$K_1 = \frac{[\text{fla}^-][\text{H}_2\text{O}]}{[\text{flaH}^-][\text{OH}^-]} \qquad (5)$$

The overall rate law can then be expressed as shown in eq 6.

$$\text{reaction rate} = k_{\text{obs}}\,[\text{OH}^-][\text{flaH}][\text{O}_2] \qquad (6)$$

By the use of the equilibrium constant K_1 in eq 4 and (5) the rate equations (3) and (6) modify to (7).

$$\text{reaction rate} = \frac{kK_1}{[\text{H}_2\text{O}]}\,[\text{OH}^-][\text{flaH}][\text{O}_2] \qquad (7)$$

The second-order rate constant k at 353.16 K and pH 10 was found to be (4.53 ± 0.07) × 10^{-2} mol^{-1} L s^{-1} (Table 1, Supporting Information). From the temperature dependence of k of the reaction the activation parameters were calculated to be $\Delta H^{\ddagger}$/kJ mol^{-1} = 59 ± 4, $\Delta S^{\ddagger}$/J mol^{-1} K^{-1} = −110 ± 11 (Figure 6, Supporting Information). The rate constant k at pH 7−8, calculated from the experimentally obtained values of k_{obs}, was in good agreement

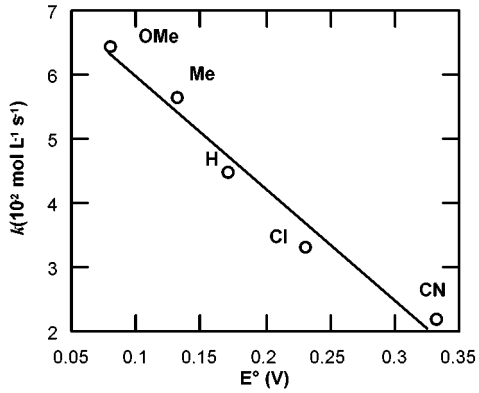


Figure 2. Plot of rate constant versus anodic oxidation potentials of 4′-substituted flavonols. Reaction conditions: 50 mL DMSO/H_2O (1:1 v/v), 80 °C, [O_2] = 1.94 × 10^{-3} mol L^{-1}, pH = 10, I = 0.1 mol L^{-1}.

with that measured at pH 10 (Table 2, Supporting Information).

Reaction rates on the oxygenation of 4′-substituted flavonols under identical conditions were also determined, and a linear Hammett plot was obtained (Figure 7, Table 4, Supporting Information). Electron-releasing substituents enhanced the reaction rate, and the reaction constant ρ was found to be −0.50. The linear correlation between the oxidation potential of flavonols and the rate constants supports that a higher electron density on the flavonolate ion makes them more nucleophilic and so the reaction easier (Table 5, Supporting Information, Figure 2), stressing its influence on the rate-determining step or possible preequilibrium. A rough but significant correlation exists between Hammett's σ values and Fieser's critical oxidation potentials.[45]

Radical Trapping and Inhibition Experiments. Since dioxygen is a diradical in its triplet ground state[46] and for the deprotonated flavonol the flavonolate ion is in its singlet state, their reaction is spin restricted.[47] However, the phenolates, diphenolates, and the flavonolate ions have a high energy HOMO orbital[45] to overcome the spin barrier and an electron transfer from the anions to O_2 will be possible. Due to their reaction, the formation of radicals (superoxide ion or flavonoxy radical) is possible. To check their presence, we carried out inhibition experiments by the use of excess 2,6-di-*tert*-butyl-4-methylphenol and 1,4-benzoquinone. Kinetic runs with these inhibitors showed no significant decrease in the reaction rates. Rate constants (k) at 353.16 K and a pH of 10 were found to be (5.15 ± 0.18) × 10^{-2} mol^{-1} L s^{-1} and (4.07 ± 0.06) × 10^{-2} mol^{-1} L $s^{-1,}$ respectively. We applied the tetrazolium blue test[48] for checking the presence of the superoxide ion. These tests were also negative, indicating that no superoxide ion is formed during the oxygenation reaction. To trap possible organic radicals, we used radical traps such as phenyl *N*-*tert*-butylnitrone and 2,6-dichloronitrosobenzene. By carrying out the reactions in the presence of these radical traps

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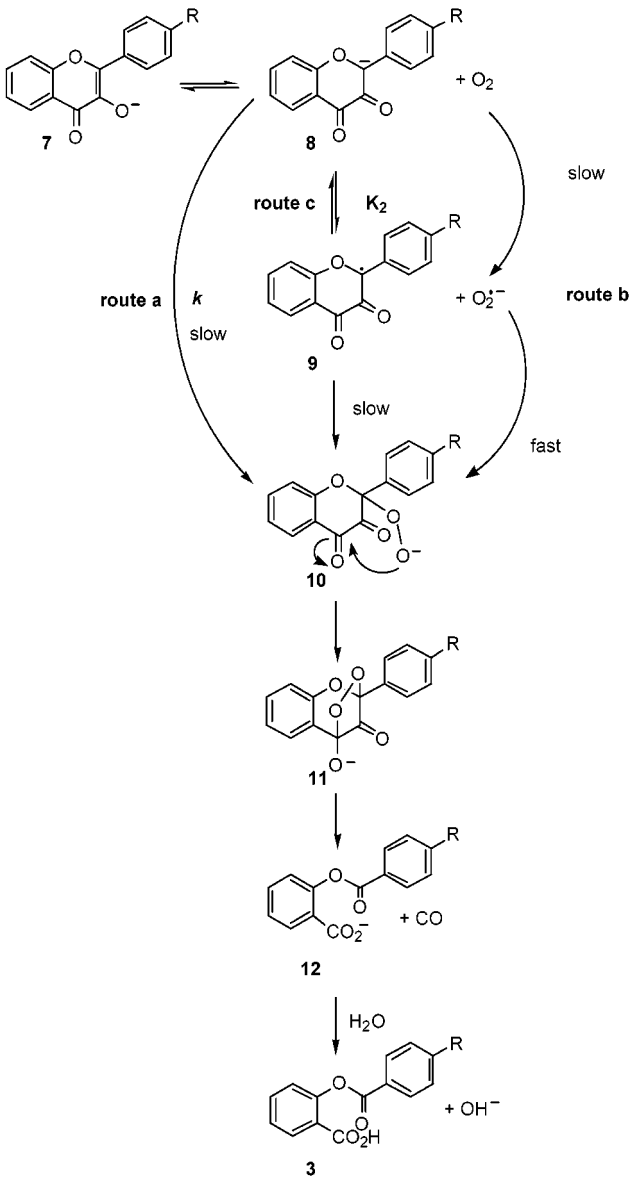
Scheme 1



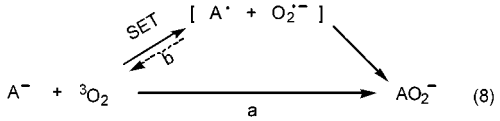
no persistent trapped radicals could be detected by EPR. These findings suggest that in the course of the base-catalyzed oxygenation of flavonols free radicals are not formed and a radical mechanism for the reaction seems unsupported.

Discussion and Conclusions

On the basis of the chemical, spectroscopic, inhibition, spin trapping, and kinetic data, suggestions can be made for the possible mechanism of the oxygenation reaction. These are summarized in eq 4 and Scheme 1. From the kinetic point of view three reaction routes seem to satisfy the kinetic fundings. According to route a, the mesomeric form **8** of the deprotonated flavonol **7** reacts with dioxygen in an electrophilic, slow rate-determining process to the deprotonated hydroperoxide species **10**. Route b involves a slow rate-determining single electron transfer from the flavonolate ion **8** to dioxygen resulting in the flavonoxy radical **9** and $O_2^{\bullet-}$. This is then followed by a radical coupling reaction between the two radicals formed (**9** and $O_2^{\bullet-}$) ending up in the peroxidic species **10**, as postulated in route a. In route c, the flavonolate ion undergoes a fast and reversible electron transfer with dioxygen yielding the flavonoxy radical **9** and superoxide ion. K_2 must be small, the equilibrium have to be mainly on the side of the starting compounds. This is followed then by the rate-determining step between the radical **9** and $O_2^{\bullet-}$. Steady-state treatment of this sequence of elementary steps assuming d[**7**]/dt = 0 leads to a second-order overall rate eq 6. Species **10** is common in each proposed pathways and also the intramolecular nucleophilic attack of the −O−O⁻ group on the 4-C=O yielding the endoperoxide **11**, which decomposes by loss of CO to the depside (*O*-benzoylsalicylic acid) in fast consecutive steps. To decide the dominant reaction pathway of the kinetically satisfying alternatives a−c, inhibition, spin trapping experiments and checking the presence of superoxide ion by nitro blue tetrazolium chloride were applied, and all these efforts turned out to be negative suggesting the absence of radical species in the reaction mixtures. EPR studies failed also to detect organic radicals. These facts seem to suggest reaction route a, in which the **8** mesomeric form of the deprotonated flavonol is attacked by O_2 in an electrophilic rate-determining reaction leading to the peroxide species **10**, as the most probable event under this conditions.

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.[49] 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 8b). Carbanions of radicals with electron affinities = 20 kcal react with ground state dioxygen while those of higher electron affinities do not, presumably because reaction 8b has become unfavorable.[50] 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. The fact that in this oxygenation reaction no radical species could be detected while in aprotic conditions at the oxygenation of potassium flavonolate the presence of the flavonoxy radical could be proved by EPR, suggest that in these reactions basically two reaction pathways (eq 8, parts a and b) are possible.

The first is a SET reaction where electron transfer from the flavonolate to $^3O_2$ is dominant, which is followed by a fast coupling reaction to $flaO_2^-$. 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 eletrophilic attack of $^3O_2$ on the anion fla^- leading directly to $flaO_2^-$. 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 in 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 decreas-

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ing it. The flavonoxy radical (fla$^{\bullet}$) 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 instability.[51] That means that reaction pathway a is strongly preferred, and the base-catalyzed oxygenation of flaH in DMSO/H_2O solvent follows exclusively this way. The very polar solvent supports an ionic mechanism while the less polar DMF makes the SET reaction also possible, where the quenching of fla$^{\bullet}$ by H_2O is not possible. This argumentation seem to be supported by results of the oxygenation of the isoelectronic 1H-2-phenyl-3-hydroxy-4-oxoquinoline (**4c**) in DMF and DMSO/H_2O solvents. The radical **4c**$^{\bullet}$ formed is stable in protic solvents and is really persistent in aprotic solvents for weeks.[52] For this reason the oxygenation of **4c** proceeds in both type of solvent via a SET reaction, due to the enhanced stability of the radical.[53]

In conclusion, it can be said that the base-catalyzed oxygenation of flavonol proceeds principally via two mechanistic pathways, a SET reaction and/or a one step electrophilic reaction of $^3O_2$ on the flavonolate ion. The spin restriction can be overcome in both pathways either by a spin change (**route b**) or by the high energy content of fla$^-$. The ratio of **route a** to **route b** depends on the stability of the radical to be formed. If A$^{\bullet}$ is stable, route b is preferred and in the case of an unstable A$^{\bullet}$ **route a** may be the main pathway. In the case of the base-catalyzed oxygenation of flavonol in DMSO/H_2O solvent **route a** seems to be the main course of the reaction. This is mainly due to the unstability of the radical fla$^{\bullet}$. Similar oxygenations of the isoelectronic 1H-2-phenyl-3-hydroxy-4-oxoquinoline (**4c**), where the radical A$^{\bullet}$ is very stable provides evidence for this assumption. Further work is in progress to clear factors influencing a stepwise (SET) or concerted pathway in similar reactions with $^3O_2$.

Experimental Section

General Procedures. All reactions, unless otherwise described, were done under an inert atmosphere of dry nitrogen using standard Schlenk-type techniques.[54] Solvents used for the reactions were purified by literature methods[55] and stored under argon. 3-Hydroxyflavone,[40] 3-hydroxy-4′-methoxyflavone,[56] 3-hydroxy-4′-methylflavone,[56] 3-hydroxy-4′-chloroflavone,[56] and 3-hydroxy-4′-cyanoflavone[56] were prepared according to literature methods. Diazomethane was freshly prepared according to the literature in ether and immediately used for the methylation reactions.[57] Infrared spectra were recorded on a Specord 75 IR (Carl Zeiss) spectrophotometer using samples mulled in Nujol between KBr plates or in KBr pellets. Electronic spectra were measured on a Shimadzu UV-160 spectrometer using quartz cells. GC analyses were performed on a HP 5830A gas chromatograph equipped with a flame ionization (TCD) detector and a CP SIL 8CB (molecula sieve 5A) column. GC−MS measurements were recorded on a HP 5890II/5971 GC/MSD spectrometer at 75 EV. CV measurements were carried out with a Pt working electrode in DMSO solution at room temperature with ca. 10^{-3} M solutions, NBu_4ClO_4 as supporting electrolyte, and scan rate 100 mV s^{-1}. The potential values are relative to the SCE using Ag/AgCl reference electrode. The concentrations of dissolved dioxygen were measured at atmospheric pressure with a Beckman Oxygen Analyzer. The sensor was calibrated with dioxygen saturated DMF.[58]

Kinetics. Kinetic measurements in 50% DMSO−H_2O solutions were carried out at 80 °C at an ionic strength of 0.1 mol L^{-1}, adjusted with KNO_3. In the pH range 7−8 a buffer of KH_2PO_4 and NaOH, between 8 and 9 a buffer of Borax and HCl, between 9 and 10.8 a buffer of $NaHCO_3$ and NaOH was used.[59] In a typical experiment, flavonol was dissolved under argon atmosphere in a thermostated reaction vessel with an inlet for removing samples with a syringe and connected to a mercury manometer to regulate constant pressure. The vigorously stirred solution was then heated to the appropriate temperature. A sample was then removed by syringe, and the initial concentration of flavonol was determined by UV−vis spectroscopy measuring the absorbance of the reaction mixture at 344 nm (log ϵ = 4.15, pH = 7−8) [λ_{max} of a typical band of flaH] or at 412.5 nm (log ϵ = 4.15, pH = 8−10.8) [λ_{max} of a typical band of fla$^-$].[60,61] The argon was then replaced by dioxygen, and the consumption of flavonol was analyzed periodically (ca. every 5−10 min). The rate of consumption was independent of the stirring rate, excluding eventual diffusion control effects. Experimental conditions are summarized in Table 1 (Supporting Information). The temperature was determined with an accuracy of ±0.5 °C; the concentrations of flaH were measured with a relative mean error of ca. ±2%; the pressure of dioxygen was determined with an accuracy of ±0.5%.

Product Composition. In a separate experiment, flavonol (0.476 g, 2 mmol) in 50% DMSO−H_2O (50 mL) at pH 10 was treated with dioxygen (0.1 MPa) at 80 °C for 20 h yielding a pale yellow solution. The mixture was then acidified with dilute hydrochloric acid and extracted with ether. The extract was dried (Na_2SO_4) and evaporated to dryness to give a residue (0.442 g). The GC−MS analysis of the residue after treatment with etheral diazomethane showed the presence of a mixture of salicylic acid and benzoic acid (1:1, 83%) and a small amount of *O*-benzoylsalicylic acid (∼2%).[43] The GC analysis of the gas phase showed the presence of 1.73 mmol CO (86%).

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Supporting Information Available: Kinetic diagrams and tables of kinetic data. This material is available free of charge via the Internet at http://pubs.acs.org.

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