On the Mechanism of a Model Quercetinase Reaction using a Cobalt Schiff-base Complex

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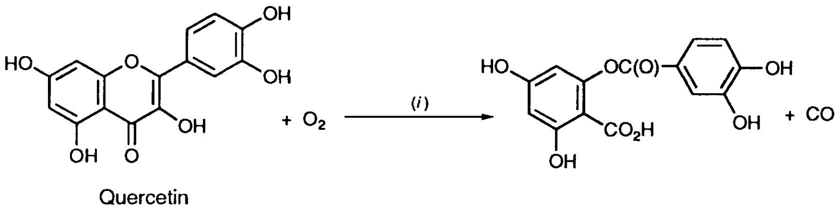
Cyclic voltammetry of a substrate anion–catalyst binary complex intermediate [Co^{III}(salen)L^1] [substrate = various flavonols (HL^1); H_2salen = *N,N'*-bis(salicylidene)ethane-1,2-diamine] in a model quercetinase reaction using [Co^{II}(salen)] as catalyst has revealed that the intermediate partly undergoes ionic dissociation in dimethylformamide (dmf) to give L^1 and [Co^{III}(salen)(dmf)$_2$]$^+$. Dioxygen is then incorporated into the flavonolate anion in a non-radical manner, which is promoted by the counter cobalt cation complex, finally to give [Co^{III}(salen)L^2(dmf)] (HL^2 = a depside product) which then forms L^2 and [Co^{III}(salen)(dmf)$_2$]$^+$. The reaction rate was affected by the nature of the cation employed: [Co^{III}(salen)(dmf)$_2$]$^+$ is more effective than K^+.

Quercetinase, a dioxygenase produced by *Aspergillus flavus*, catalyses the total insertion of oxygen into the substrates quercetin [2-(3,4-dihydroxyphenyl)-3,5,7-trihydroxy-4*H*-benzopyran-4-one] and related 3-hydroxyflavones, resulting in oxidative cleavage of the heterocyclic ring to give the corresponding depsides and carbon monoxide (Scheme 1).[1] The catalytic centre of this dioxygenase contains a Cu^{2+} ion, but no information about the nature and role of the central metal ion has yet been obtained.[1] Studies on structural and functional models are important in order to elucidate the mechanism of the enzyme reaction.

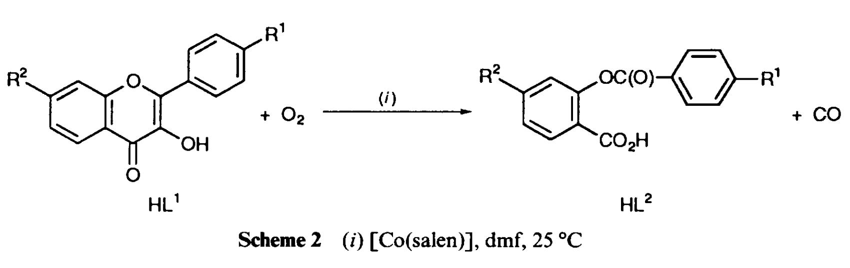
The photosensitized oxygenation of 3-hydroxyflavone (3-hydroxy-2-phenyl-4*H*-benzopyran-4-one),[2] the first reaction, resulted in oxygenolysis of its heterocyclic ring without quantitative formation of carbon monoxide, suggesting that the mechanism of the photooxygenation is different from that of the enzyme reaction. The dioxygenolysis of 3-hydroxyflavones HL^1 (R^1 = H, OH or OMe; R^2 = H) promoted by a strong base such as potassium *tert*-butoxide in dimethylformamide (dmf) gives the same products as those produced in the enzyme reaction, but when a hydroxy group is substituted at the 7 position the reaction becomes complicated, giving a mixture of many products.[3,4]

Investigations on the oxygenation of compounds HL^1 catalysed by copper(II) complexes have been reported. Unfortunately, however, good selectivity was not observed for the Cu^{2+}-catalysed oxidations.[5,6] On the contrary, catalysis by [Co(salen)] [H_2salen = *N,N'*-bis(salicylidene)ethane-1,2-diamine] leads to highly selective oxygenolysis to give the corresponding depsides HL^2 and carbon monoxide in excellent yields even when a hydroxy group is substituted at the 7 position in HL^1 (Scheme 2).[7] The [Co(salen)]-catalysed oxygenation was accelerated when a hydroxy or methoxy group was substituted at the 7 or 4' position in HL^1, in the order OH > OMe; 4'-OH > 7-OH. A similar substituent effect has been observed for the enzyme reaction[1] as well as the base-promoted oxygenation of HL^1,[3,4] suggesting that a common mechanism must be operating in these reactions. Therefore, it seems important to clarify the mechanism of the [Co(salen)]-catalysed oxygenation.

Recently, we have found that the reaction of [Co^{III}(salen)(OH)] **1a** with HL^1 (R^1 = R^2 = H; R^1 = OMe, R^2 = H) gives flavonolatocobalt(III) complexes **2a** and **2b** (Scheme 3) which are susceptible to oxygenation in a co-ordinating solvent such as pyridine (py) or dmf, but quite stable to oxygen in non-co-ordinating solvents.[8] The crystal structure determination of **2a** revealed that the substrate anion co-ordinates as a bidentate ligand accompanied by a conformational change of the salen ligand.[9] The bidentate structure of the substrate is maintained in dichloromethane, but in dmf the flavonolate behaves as a



Scheme 1 (*i*) Quercetinase (Cu^{2+})



Scheme 2 (*i*) [Co(salen)], dmf, 25 °C

monodentate ligand due to ligation of the solvent as judged by ^{1}H NMR spectroscopy.[8] Therefore, the co-ordinatively saturated complex **3** should be the oxygen-sensitive intermediate.

The question arises as to how dioxygen is incorporated into the active intermediate. Since direct dioxgyen insertion into complex **3** is not likely,[10] one must assume the reaction of dioxygen either with a flavonoloxy radical or a flavonolate anion, arising from homolytic or heterolytic cleavage of the flavonolate–cobalt bond in **3**, respectively.

The present paper deals with the detailed mechanism of the [Co(salen)]-catalysed highly selective oxygenolysis of HL^{1} in dmf studied by means of cyclic voltammetry. A non-radical bimolecular reaction between dioxygen and a free flavonolate anion with assistance from a cationic complex [Co^{III}(salen)(dmf)$_2$]$^{+}$ [11] is discussed.

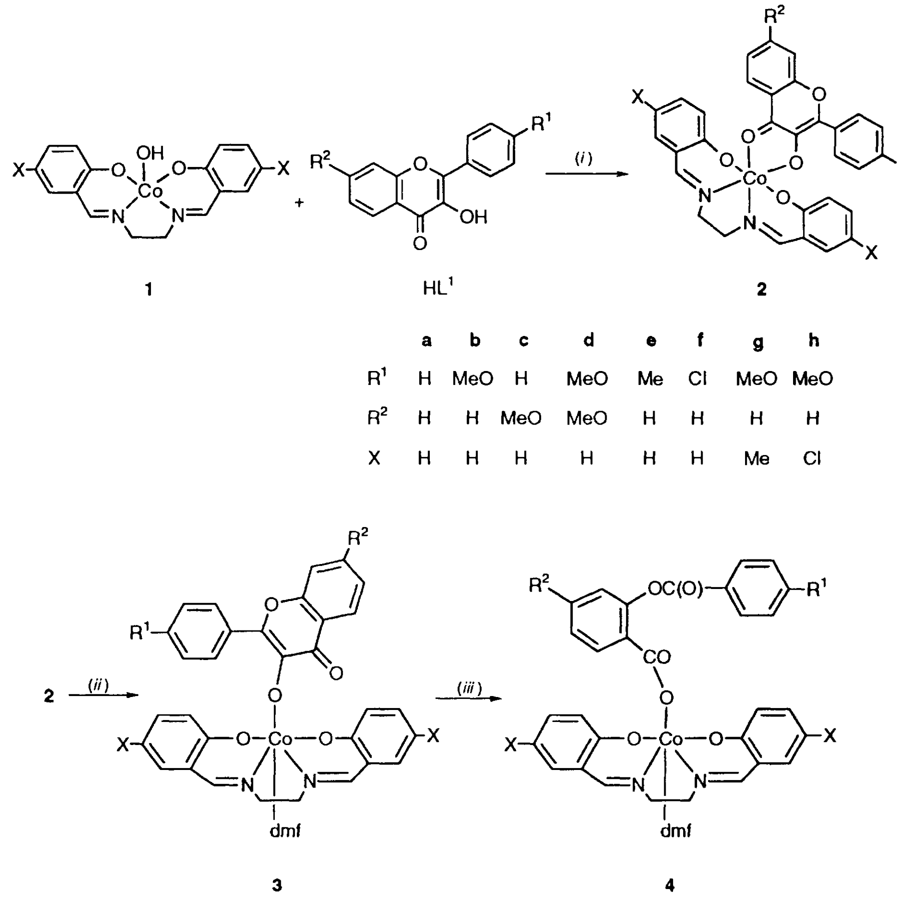
Results and Discussion

It has been shown that the monodentate flavonolato complex **3b** is thermally stable. No dimeric product arising from a flavonoloxyl radical was obtained. The results clearly indicate that no homolytic cleavage of the flavonolate–cobalt bond in **3b** takes place. In other words, no electron transfer occurs from the flavonolate anion to the cobalt(III) centre. Nevertheless, when oxygen was bubbled through a solution of **2b** in dmf or in dichloromethane containing pyridine, dioxygenation occurred to give the Co^{III}(salen)(dmf) complex **4b**.[8]

Cyclic Voltammogram of Complex **2** *in dmf.*—A solution of complex **2b** (5×10^{-4} mol dm^{-3}) in dmf containing NBu$_4$PF$_6$ (0.1 mol dm^{-3}) under nitrogen showed very weak redox currents in an electrode system comprised of Pt (working electrode)–Pt (counter electrode)–Ag–AgNO$_3$ in MeCN (reference electrode). However, oxygenation of this solution resulted in an increase in peak currents to give the same quasi-reversible curve as that observed for [Co^{II}(salen)] itself (Fig. 1).[12]

If these results are for compound **2b** or **3b** itself, the formal potential $E°$(Co^{II}–Co^{III}) should be influenced by substituents R^{1} and R^{2} in **2**, because an electron-releasing group at the 4′ or 7 position is expected to increase the electron density at the cobalt centre. Thus, the cyclic voltammetric behaviour of a series of compounds **2** in dmf was examined. When an oxidation current was applied by sweeping the potential from negative to positive no oxidation peak (Co^{II} to Co^{III}) was observed, but the reverse sweep gave a quasi-reversible curve for the Co^{III}–Co^{II} redox couple. The observed formal potentials $E°$ of −447 (**2a**), −447 (**2b**), −446 (**2c**), −444 (**2d**), −447 (**2e**) and −456 mV (**2f**) are almost the same as that of [Co(salen)] **1** (X = H) −451 mV determined in this work (*cf.* −466 mV[12]). Furthermore, the $E°$ values in dmf of complexes **2g** (−452) and **2h** (−412 mV), in which 5 and 5′ positions of the salen ligand are substituted with Me and Cl groups, respectively, were almost identical with those of the corresponding parent Co(salen) derivatives **1** (X = Me, −447; Cl, −421 mV determined in this work).

These results strongly suggest that the observed formal potentials of complexes **2** must be assigned to those of **1**, indicating that compounds **3** formed *in situ* are dissociated in dmf to generate the corresponding cobalt(III)–Schiff base cation. Thus, the small peak current (i_p) under nitrogen [Fig. 1(*a*)] is rationalized by assuming that only a small amount of **3b** is dissociated. Fig. 1(*b*) shows the time course of the changes observed by repeating the cyclic voltammetric measurement under nitrogen after oxygen bubbling (10 min, with disconnection of the electrode) through the solution in Fig. 1(*a*). The value of i_p increased with time. The results suggest that **3b** is gradually oxygenated to **4b**, the cyclic voltammogram [Fig. 1(*d*)] of which was identical with that of the final oxygenated



	a	b	c	d	e	f	g	h
R^{1}	H	MeO	H	MeO	Me	Cl	MeO	MeO
R^{2}	H	H	MeO	MeO	H	H	H	H
X	H	H	H	H	H	H	Me	Cl

Scheme 3 (*i*) CH$_2$Cl$_2$; (*ii*) dmf; (*iii*) O$_2$

solution of **3b**. Since the cyclic voltammogram did not change with time, which indicates a very rapid ionic dissociation equilibrium of **4b** giving rise to L^{2b} and $[Co^{III}(salen)(dmf)_2]^+$, the time-dependent change seen in Fig. 1(*b*) should correspond to the rate of oxygenation of **3b**. Actually, similar time-dependent changes with different rates were observed for compounds **2a**–**2f** in dmf (Fig. 2).

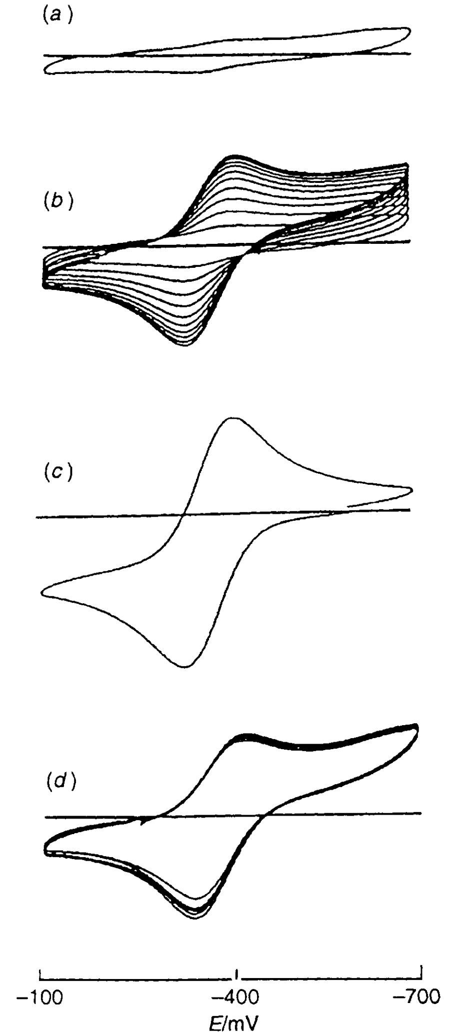


Fig. 1 Cyclic voltammograms of compounds (1 mmol dm^{-3}) in dmf containing NBu_4PF_6 (0.1 mol dm^{-3}) at a platinum electrode with a scan rate of 100 mV s^{-1} under nitrogen: (*a*) **2b**, (*b*) after oxygenation of (*a*) as described in the text, (*c*) [Co(salen)], and (*d*) **4b**; no time-dependent change of i_{pa} is observed

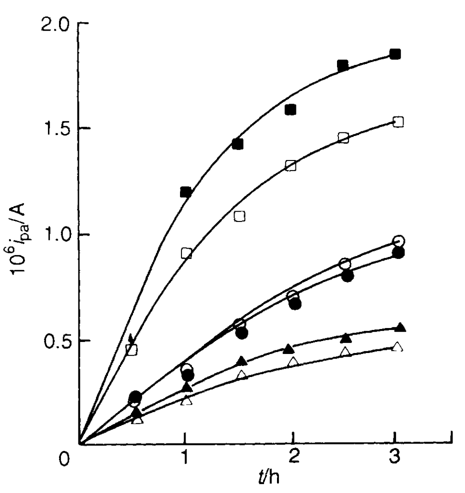


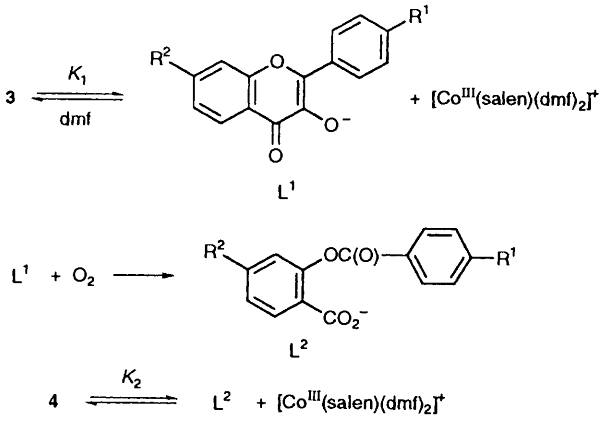
Fig. 2 Time-dependent change of i_{pa} for complexes **2** in dmf under conditions similar to those in Fig. 1 (*b*): (■) **2d**, (□) **2b**, (○) **2c**, (●) **2e**, (▲) **2a** and (△) **2f**

As seen from Fig. 2, the substituent effect of the flavonolate ligand in complexes **3** on the increase in i_p is in the order 4′-MeO > 7-MeO ≈ 4-Me > 4-H > 4-Cl, which is in accord with that observed for the base-promoted oxygenation of HL^1.[3] These findings also support the dissociation of **3** in dmf to generate a free flavonolate anion L^1 and $[Co^{III}(salen)(dmf)_2]^+$,[11] although no oxidation peak current for the free flavonolate anion was observed. These results are represented in Scheme 4, where equilibrium constant K_2 is much larger than K_1.

Therefore, we have examined the cyclic voltammetry of compound HL^{1b} in the presence of potassium *tert*-butoxide in dmf in order to confirm whether the oxidation peak current for flavonolate anion can be observed or not. It was found that only an anodic peak current (i_{pa}) appeared [Fig. 3(*a*)] at a position very close to the potential (E_{pa}) for [Co(salen)]. When oxygen was bubbled through the above solution of HL^{1b} for 10 min, followed by cyclic voltammetry under nitrogen i_{pa} decreased. Repeating the same procedure resulted in a time-dependent decrease in i_{pa} [Fig. 3(*a*)], indicating oxygenation of the substrate anion, where no redox potential for the product anion was observed.

The formal potential of cobalt Schiff-base complexes is known to undergo a negative shift when a strong donor ligand is added.[11,13] Thus, the addition of an excess of pyridine to a solution of **2b** in dmf promoted the dissociation of the resulting **3b** and the cyclic voltammogram of the Co(salen) moiety was shifted to a much more negative position, while the E_{pa} of the flavonolate anion L^{1b} stayed at the original position [compare Figs. 3(*a*) and 3(*b*)]. The new cobalt species is assigned as $[Co(salen)(py)_2]^+$.[13] Further, the peak currents increased with time [Fig. 3(*b*)]. This suggests that the ionic dissociation of **3b** is slow. After the i_{pa} of L^{1b} had reached its maximum, oxygen was bubbled (10 min) through the solution (with disconnection of the electrode) and then measurements were made under nitrogen. A decrease in i_{pa} was observed. Upon repeating the procedure, the i_{pa} of L^{1b} decreased with time, while that of $[Co^{III}(salen)(py)_2]^+$ was unchanged [Fig. 3(*c*)]. These results provide good evidence that the oxygenation of **3** occurs only after dissociation has taken place (Scheme 5).

Structural Requirements of Cobalt Schiff-base Complexes for Catalysis.—In order to find out whether the redox potential



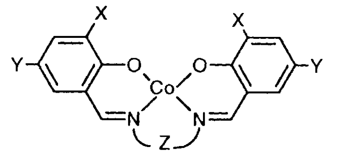
Scheme 4

$$3 \xrightarrow{(i)} L^1 + [Co^{III}(salen)(py)_2]^+$$

$$L^1 + O_2 \longrightarrow L^2$$

Scheme 5 (*i*) py, dmf

Table 1 Redox potentials and catalytic activity of cobalt Schiff-base complexes **5**



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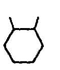
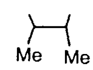
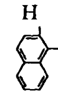
	a	**b**	**c**	**d**	**e**	**f**	**g**	**h**
X	H	H	H	H	H	Bu^t	Bu^t	Bu^t
Y	H	H	MeO	Me	Bu^t	H	MeO	Me
Z	1,2-C_6H_4	$(CH_2)_2$	$(CH_2)_2$	$(CH_2)_2$	$(CH_2)_2$	$(CH_2)_2$	$(CH_2)_2$	$(CH_2)_2$
$E^{\circ a}$/mV	—	−451	−432	−464	−452	−432	−408	−426
$10^{-7}\ v^b$/mol dm^{-3} s^{-1}	55.0	7.0	7.0	6.5	6.5	9.9	9.9	9.9

	i	**j**	**k**	**l**	**m**	[Co(tpp)]
X	Bu^t	H	H	H	H	—
Y	Cl	H	H	H	H	—
Z	$(CH_2)_2$	 Me Me Me Me		 Me Me		
$E^{\circ a}$/mV	−421	−343	−361	−361	−309	
$10^{-7}\ v^b$/mol dm^{-3} s^{-1}	7.4	4.2	4.2	1.8	0.2	22

^{a} Formal potentials were determined under the same conditions as those given earlier.[12] ^{b} The reaction rate, determined from the time course of the consumption of the starting material by means of a TLC analyser.

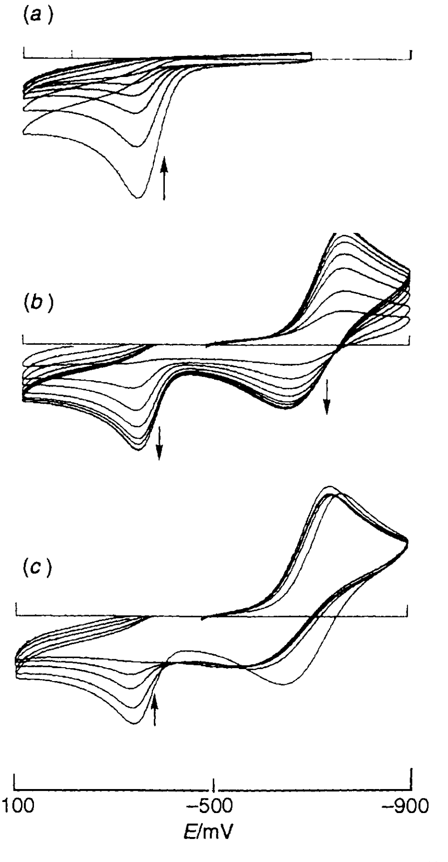


Fig. 3 Time-dependent change of i_{pa}: (*a*) compound HL^{1b} in the presence of KOBu^t in dmf under the conditions given in the text, (*b*) time course for complex **3b** in the presence of pyridine (10^3 equivalents) in dmf under nitrogen, and (*c*) after the oxygenation of the final solution obtained in (*b*) under conditions described in the text

(E°) of the cobalt catalyst is important for the catalysis, the oxygenation of HL^{1b} with various cobalt Schiff-base complexes [Co(sb)] **5** and [Co(tpp)] (H_2tpp = 5,10,15,20-tetraphenylporphyrin) in dmf was examined. It was found that compound HL^2 (R^1 = OMe, R^2 = H) was the sole product in all cases and there was almost no correlation between the catalytic activity and E° (**5b**–**5i**) (Table 1).

Among the complexes examined, **5a** and [Co(tpp)], in which the planarity of the ligands is fixed, showed the highest catalytic activity. Any other modification of the amine part in the Schiff base in **5** shifted E° to values more positive than that of [Co(salen)] **5b** but retarded the reaction (**5j**–**5m**). The higher catalytic activity of **5a** was attributed to the higher concentration of flavonolate resulting from rapid ionic dissociation of the flavonolato complex of type **3** as judged by cyclic voltammetry (Fig. 4). The concentration of L^{1b} generated from **3** (1 mmol dm^{-3}) formed *in situ* from an equimolar mixture of HL^{1b} and **5a** in dmf was about 0.25 mmol dm^{-3} as estimated by comparison of the i_{pa} with that for the KOBu^t–dmf system. Fig. 5 shows a comparison of the time course of the oxygenation of HL^{1b} with **5a** and that with KOBu^t in dmf. Taking into account the concentration of L^{1b} in both cases ($[L^{1b}]_{Co}$ is less than $\frac{1}{4}$ $[L^{1b}]_K$ estimated by comparison of i_{pa} values), the reaction rate with the cobalt complex is about ten times faster than that with KOBu^t. The results indicate that the oxygenation rate is regulated by the nature of the counter metal cation.

Mechanism.—From the above observations a reasonable mechanism for the present model quercetinase reaction can be deduced (Scheme 6). The initial catalytically active species in dmf is [Co^{III}(sb)(OH)(dmf)]. Then, the hydroxo complex reacts with HL^1 to give **3**, which is dissociated to flavonolate anion L^1 and [Co^{III}(sb)(dmf)$_2$]$^+$. The final step involves dioxygen incorporation into the flavonolate anion in a non-radical bimolecular manner under the influence of the counter metal cation species. In general, such a non-radical bimolecular reaction between a singlet substance and the ground state of molecular oxygen (triplet) giving rise to a singlet product state is normally forbidden because of the well known spin-conservation rule.[14] However, there are some other examples of oxygenation reactions using the ground state of dioxygen, where a non-radical bimolecular reaction mechanism is considered reasonable.[15–19] For these non-radical dioxygenation reactions a mechanism involving intersystem crossing between a triplet

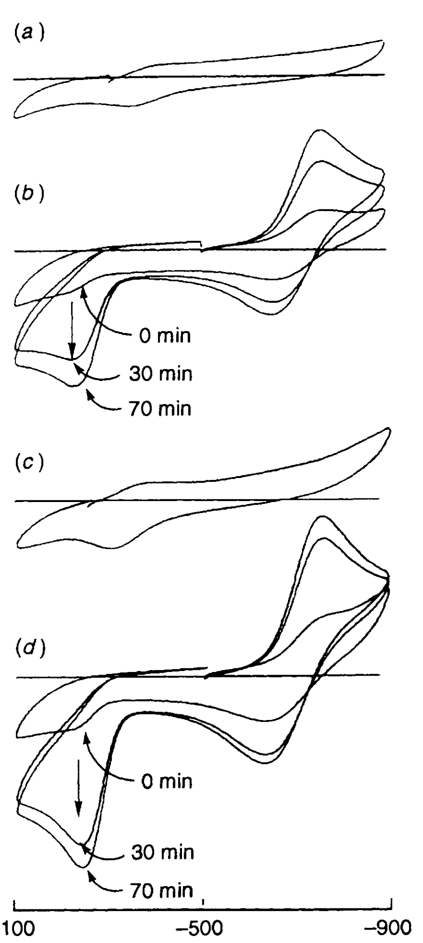


Fig. 4 Comparison of magnitude of i_{pa} for the flavonolato complexes of type 3 derived from HL^{1b} and (*a*) **5b** and (*c*) **5a** in dmf under nitrogen; (*b*) and (*d*) after addition of py (500 equivalents) to each solution

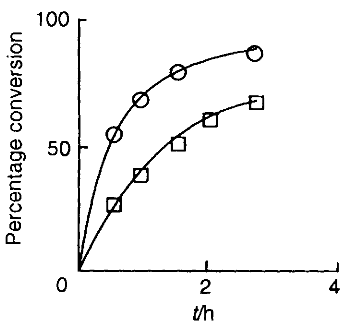


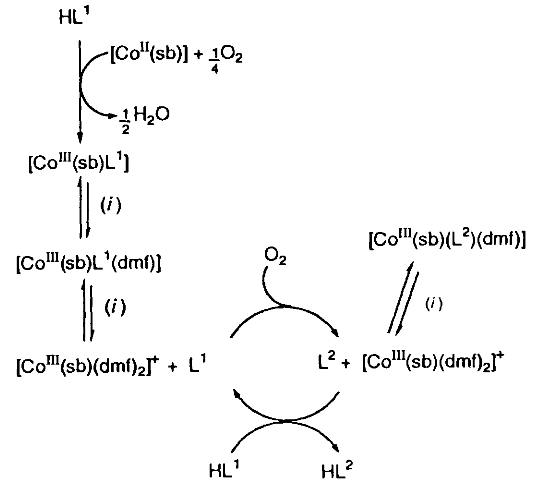
Fig. 5 Time course of the oxygenation of (○) complex **2b** (1 mmol) and (□) HL^{1b} (1 mmol) with $KOBu^t$ (1 mmol) in dmf (20 ml) at 25.0 ± 0.1 °C

charge-transfer complex ^{3}(c.t.) and a singlet one ^{1}(c.t.) has been proposed (Scheme 7).[20,21] It is reasonably assumed that the acceleration of the intersystem crossing by $[Co^{III}(salen)(dmf)_2]^+$ is ten times greater than that by K^+.

Experimental

All melting points are uncorrected. Elemental analyses were performed with a Yanaco CHN Corder MT-5 analyser. Infrared spectra were recorded on a JASCO FT/IR-500 spectrophotometer, ^1H NMR spectra on JEOL PMX60si and Varian Unity 300 spectrometers.

Flavonols HL^1 except HL^{1a} which is commercially available, were synthesised according to the literature methods: HL^{1b},[22] HL^{1c},[23] HL^{1d},[23] HL^{1e}[22] and HL^{1f}.[22]

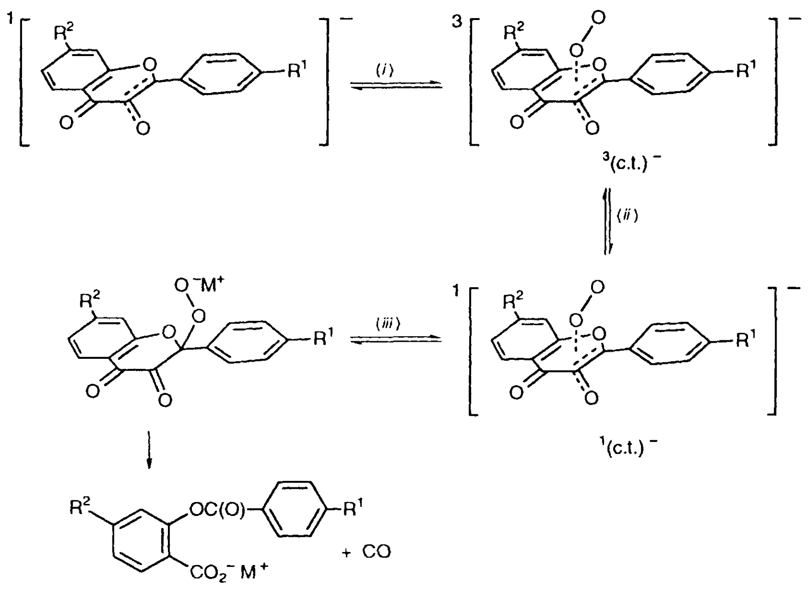


Scheme 6 HL^1 = Flavonol substrate, HL^2 = depside product, sb = Schiff-base dianion. (*i*) dmf

Synthesis of Flavonolatocobalt(III) *Complexes* **2**.—A suspension of $[Co(salen)(OH)]$[24] (2.3 mmol) and an appropriate flavonol HL^1 (11.6 mmol) in dichloromethane (250 cm^3) was stirred at room temperature for 5 h. The mixture was filtered and the filtrate evaporated to 100 cm^3. To the resulting solution was added diethyl ether to give yellow-green fine crystals, which were collected on a funnel and purified by reprecipitation: addition of ethyl acetate to a dichloromethane solution. The following analytical data were obtained: **2a**, yield 82.8 (Found: C, 66.4; H, 4.10; N, 4.75. $C_{31}H_{23}CoN_2O_5$ requires C, 66.2; H, 4.10; N, 5.00%); **2b**, yield 83.8 (Found: C, 65.0; H, 4.15; N, 4.55. $C_{32}H_{25}CoN_2O_6$ requires C, 64.85; H, 4.25; N, 4.75); **2c**, yield 75.8 (Found: C, 62.45; H, 4.05; N, 4.80. $C_{32}H_{25}CoN_2O_6{\cdot}1.5\ H_2O$ requires C, 62.5; H, 4.50; N, 4.55); **2d**, yield 89.4 (Found: C, 62.7; H, 4.20; N, 4.35. $C_{33}H_{27}CoN_2O_7$ requires C, 62.7; H, 4.55; N, 4.45); **2e**, yield 87.4 (Found: C, 66.55; H, 4.20; N, 4.75. $C_{32}H_{25}CoN_2O_5$ requires C, 66.65; H, 4.35; N, 4.85); **2f**, yield 67.1 (Found: C, 62.1; H, 3.40; N, 4.30. $C_{31}H_{22}ClCoN_2O_5$ requires C, 62.4; H, 3.70; N, 4.60); **2g**, yield 95.0 (Found: C, 65.75; H, 4.40; N, 4.15. $C_{34}H_{29}CoN_2O_6{\cdot}0.25\ H_2O$ requires C, 65.35; H, 4.70; N, 4.50); **2h**, yield 95.3% (Found: C, 58.55; H, 3.40; N, 4.55. $C_{32}H_{23}Cl_2CoN_2O_6$ requires C, 58.1; H, 3.50; N, 4.25%).

Synthesis of the Product (*depside*) *Anion Cobalt*(III) *Complex* **4b**.—Through a solution of complex **2b** (0.44 g, 0.75 mmol) in dmf (40 cm^3) was bubbled oxygen at room temperature for 5 h. The resulting mixture was concentrated *in vacuo* [25 °C, 10 mmHg (1330 Pa)] to 10 cm^3. Addition of ether gave light brown precipitates, which were collected on a funnel, washed with ethyl acetate, and dried to give complex **4b** (79.1% yield) (Found: C, 60.9; H, 4.40; N, 5.90. $C_{34}H_{32}CoN_3O_8$ requires C, 61.0; H, 4.80; N, 6.30%).

Determination of Formal Potentials, E°.—The formal potentials of the appropriate compounds were determined from cyclic voltammograms in dmf–0.1 mol dm^{-3} NBu_4PF_6 as the mean of the oxidation and reduction peak potentials, E_p^{I} and E_p^{II}, at a platinum-disk working electrode. All potentials are given relative to an external ferrocene–ferrocenium reference. The values of E_p^{I} and E_p^{II} were obtained at room temperature at scan rates v = 50, 100 and 200 mV s^{-1}. In most cases, with cobalt complexes, nearly reversible voltammograms were obtained, but for flavonolate anions only oxidation potentials were observed.



Scheme 7 (*i*) Triplet O_2; (*ii*) intersystem crossing; (*iii*) M^+

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (No. o4225233).

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Received 24*th June* 1993; *Paper* 3/03629D