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Radical-initiated oxygenation of flavonols by dioxygen

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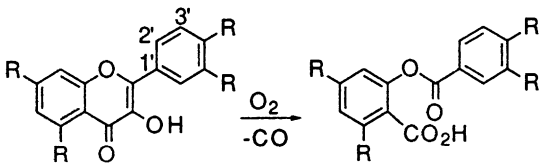
Abstract

In the presence of free radicals, such as 2,2,6,6-tetramethyl-1-piperidinyloxyl (TEMPO) and 2,6-di-*tert*-butyl-a-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-*p*-tolyloxyl (galvinoxyl), flavonols undergo catalytic oxygenation to the corresponding depsides (phenolic carboxylic acid esters), with concomitant evolution of CO. The oxygenolysis was performed in aprotic solvents (DMF, MeCN) and was followed by Glc. The results of oxygenation of 4′-substituted flavonols show that the formation of flavonoxy species is the key step in the activation process of the substrate, and that electron-releasing substituents enhance the reaction rate.

Keywords: Quercetinase; Flavonol oxidation; TEMPO; Galvinoxyl; Dioxygenase model

1. Introduction

Fungi such as *Aspergillus* or *Pullularia* species catalyze the oxidative degradation of quercetin (**1a**) to a depside (phenolic carboxylic acid ester, (**2a**) with concomitant evolution of carbon monoxide (Eq. (1)) [1]. The copper-containing metalloenzyme quercetin 2,3-dioxygenase is responsible for this reaction [2]. The quercetin is believed to bond to the copper ion through its 3-OH and 4-CO groups, but the role of the redox metal copper and its ligand environment is not yet known. Does the copper ion act as a base or its redox property is utilized? In order to understand the mechanism of this reaction, we have previously carried out autoxidation studies on potassium salts of 4′-substituted flavonols (**2b**) [3,4], and on (flavonolato)copper complexes [5–9] in aprotic and protic solvents. The mechanism of the above systems is different, however, flavonoxy radicals (**3**) seem to be the key intermediates in the reactions. In order to disclose a possible radical pathway of the mechanism of these reactions, we carried out studies on free radical-mediated oxygenation on 4′-substituted flavonols.



1 **a** R = OH **b** R = H **2** (1)

2. Experimental

2.1. Instrumentation

IR spectra were recorded in either Nujol mulls or KBr pellets on a Specord IR-75 (Carl Zeiss)

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spectrometer. GC analyses were performed on HP 5830A and HP 5890 gas chromatographs equipped with a flame ionization detector (CP SIL 8CB column), and a thermal conductivity detector (molecular sieve 5 Å column). GC-MS measurements were recorded on a HP 5890II/5971 GC/MSD at 75 eV.

2.2. Materials and methods

Flavonol [10], 4′-cyanoflavonol [10], 4′-methylflavonol [11], and 4′-chloroflavonol [11] were prepared by literature methods. Acetonitrile, DMF and diethyl ether were purified in the usual manner [12] and stored under argon. The compounds TEMPO and galvinoxyl were obtained from Aldrich and used without further purification. Diazomethane [13] was freshly prepared according to the literature in ether and immediately used for the methylation reactions. Gaseous oxygen from Messergriesheim was 99.6% and passed through P_2O_5 and Blaugel in order to remove traces of water and other impurities.

Oxygenation reactions were performed in a thermostatted reaction vessel with inlets for taking samples by a syringe, and connected to a mercury manometer to regulate constant pressure. The rate of oxygenation was independent of the stirring rate, excluding eventual diffusion control effects. GC analyses were performed after methylation with diazomethane.

3. Results and discussion

Bulk oxygenation of flavonols (**1**) with dioxygen in MeCN at elevated temperature in the presence of free radicals such as TEMPO and galvinoxyl resulted in the formation of the corresponding depsides, *O*-benzoylsalicylic acid (**2**) and carbon monoxide. The dioxygen uptake was followed volumetrically at constant pressure, where the formation of an equivalent amount of CO just compensated the amount of dioxygen consumed. The reaction was also carried out in DMF solution at ambient condition at 1:5 TEMPO to flavonol ratio and the reaction rate was found to be higher than in CH_3CN. Experimental conditions and conversions are summarized in Table 1.

It can be seen that there are no significant differences between the radical initiators TEMPO and

Table 1
Results of the TEMPO- and galvinoxyl-catalyzed oxygenation of flavonols

Substrate	Solvent	Time (h)	Catalyst	Ratio (4′R-flaH:radical)	Conversion[a] (%)
4′H-flaH	MeCN	5	TEMPO	1:0.1	5.0
	MeCN	5	TEMPO	1:0.2	10.0
	MeCN	5	TEMPO	1:0.5	26.0
	MeCN	5	Galvinoxyl	1:0.5	31.5
	MeCN	5	TEMPO	1:1.0	81.0
	DMF	5	TEMPO	2.5:0.5	18.8[b]
	DMF	10	TEMPO	2.5:0.5	32.2[b]
	DMF	15	TEMPO	2.5:0.5	58.7[b]
	DMF	5	TEMPO	2.5:0.5	4.0[c]
	DMF	10	TEMPO	2.5:0.5	8.1[c]
	DMF	15	TEMPO	2.5:0.5	9.6[c]
4′Me-flaH	MeCN	5	Galvinoxyl	1:0.5	71.3
	MeCN	5	TEMPO	1:0.5	80.4
4′Cl-flaH	MeCN	5	TEMPO	1:0.5	12.5
	MeCN	5	TEMPO	1:1.0	26.2
	MeCN	5	Galvinoxyl	1:0.5	11.5
4′CN-flaH	MeCN	5	TEMPO	1:0.5	12.0
	MeCN	5	Galvinoxyl	1:0.5	8.9

[a] In a typical experiment, 1 mmol of substrate and 0.5 mmol of free radical in 30 ml of MeCN were magnetically stirred at 70°C for 5 h. Conversion was determined by Glc after methylation with CH_2N_2.

[b] An amount of 2.5 mmol of a substrate and 0.5 mmol of TEMPO in 10 ml DMF were magnetically stirred at 25°C.

[c] Air was used instead of O_2.

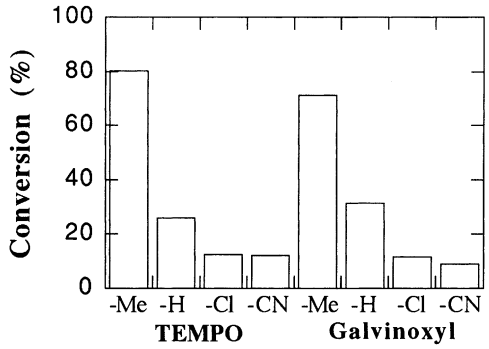

Fig. 1. Substituent effect for the TEMPO- and galvinoxyl-initiated oxygenation of 4′-substituted flavonols ($[rad^{\bullet}]_0 = 1.67 \times 10^{-2}$ M, $[4'R\text{-}flaH]_0 = 3.33 \times 10^{-2}$ M, $T = 70°C$, 30 cm^3 MeCN).

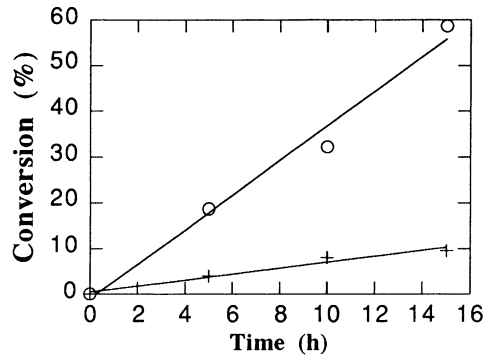

Fig. 2. The yield vs. time plots of the TEMPO-catalysed oxygenation of flavonol by O_2 (○) and air (+) (2.5 mmol substrate and 0.5 mmol TEMPO in 10 ml DMF at 25°C).

galvinoxyl, and that electron-releasing substituents on the substrate enhanced the reaction rate (Fig. 1).

The data suggest that the reaction rate is not dependent on the flavonol concentration, while higher TEMPO and dioxygen concentrations give higher reaction rates (Fig. 2).

Flavonol has been found earlier to undergo oxidative dimerization in the presence of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone [14] or MnO_2 [15] to give a C–O-coupled dehydro dimer as a result of hydrogen abstraction and coupling. The above reactions were explained by the combination of the keto and enol form of the dehidrogenated flavonol. In the TEMPO- and galvinoxyl-containing systems, the presence of dehydro dimer could not be detected. Characterization of the reaction products are summarized in Table 2.

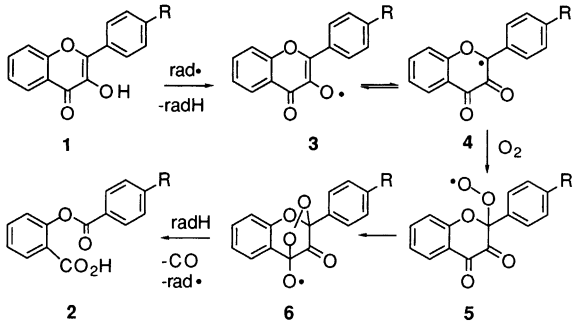

Fig. 3. The proposed reaction mechanism for the radical-initiated oxygenation of flavonols ($rad^{\bullet}$ = TEMPO or galvinoxyl).

On the basis of the above results, the mechanism shown in Fig. 3 can be proposed for the radical-initiated oxygenation of flavonol (**1**). We believe that in the presence of free radicals, the first step is the formation of the flavonoxy radical (**3**) in a fast step via hydrogen abstraction. Thereafter, the keto form of the flavonoxy radical (**4**) reacts with the biradical dioxygen in a rate-determining radical–radical coupling step forming the peroxy radical (**5**) and then the endoperoxide (**6**). The endoperoxide decomposes then in a fast step to give *O*-benzoylsalicylic acid (**2**) after hydrogen abstraction and so closes the catalytic cycle.

The results outlined above suggest clearly that the oxygenation of flavonols via a radical reaction pathway is right feasible, and can not be excluded

Table 2
Spectral data of products (4′R-*O*-bsH)

Products	GC-MS (*m*/*z*)	IR (cm^{-1})
4′H-*O*-bsH	256 (M^+, 3), 225 (1), 105 (100)	1739, 1700
4′Me-*O*-bsH	270 (M^+, 5), 239 (2), 119 (100)	2864, 1737, 1684
4′Cl-*O*-bsH	290 (M^+, 8), 259 (4), 139 (100)	1743, 1687
4′CN-*O*-bsH	281 (M^+, 11), 208 (6), 139 (100)	2227, 1720, 1707

from the list of possible ways of flavonol oxygenations including the enzymatic reaction as well.

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