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## Phenoxy Radicals: Flavonoids

by

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### Abbreviations:

- BHA: benzhydroxamic acid
- BHT: butylated hydroxytoluene
- $\Delta H$ : enthalpy
- $\Delta S$ : entropy
- $e_{aq}^-$ : hydrated electron
- ROS: reactive oxygen species
- UV: ultra violet
- $E_7$ : redox potential (volts)
- $k$ : rate constant

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### **Abstract**

Phenoxy radicals contain one or more unpaired electrons delocalized into the benzene ring. This paper focuses on the flavonoids and its different classes. Flavonoids are group of polyphenolic compounds, diverse in chemical structure and characteristics. They are found ubiquitously in plants and are potent antioxidants, free radical scavengers, metal chelators and inhibit lipid peroxidation.

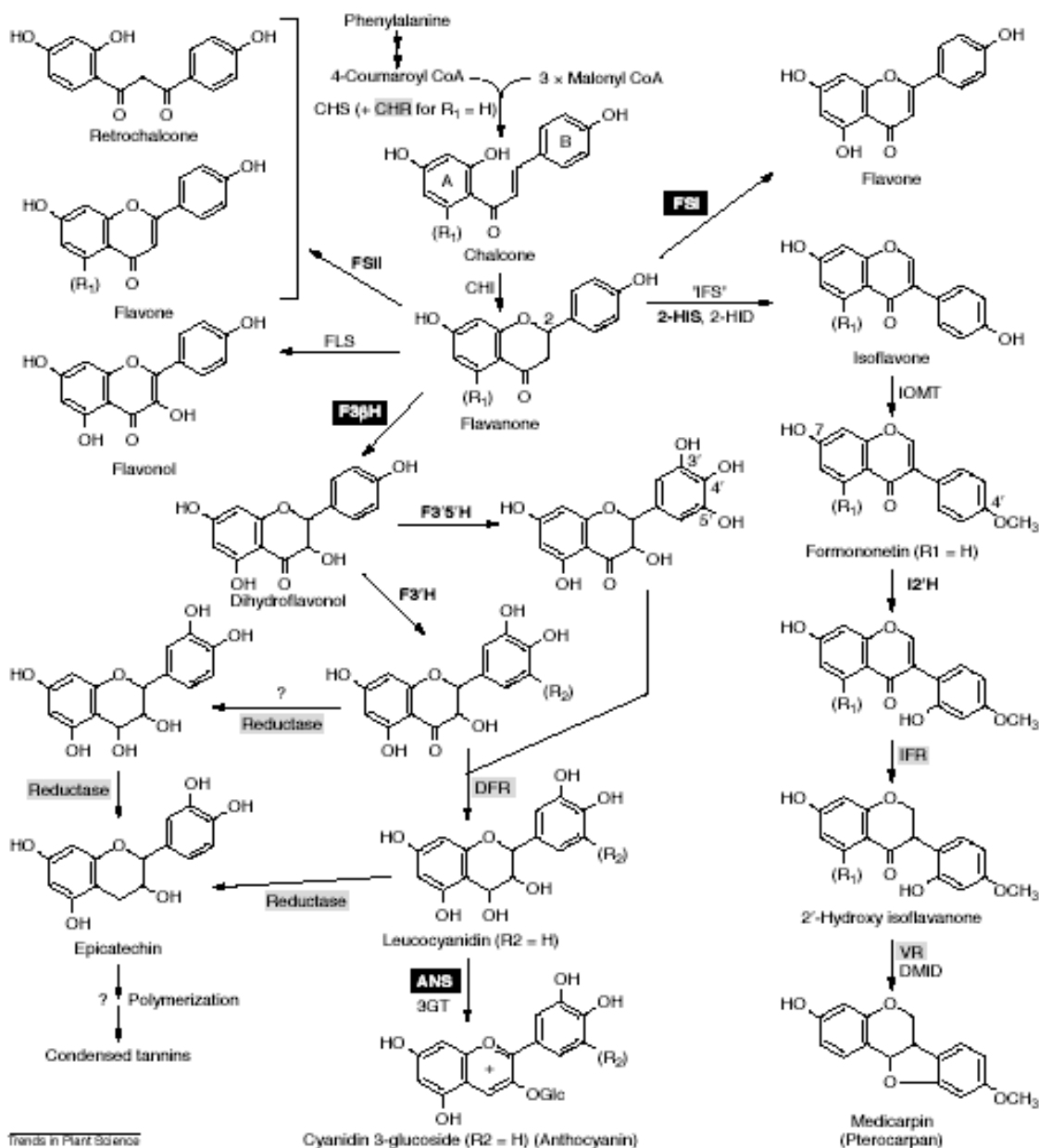
## Introduction

Phenoxy radicals are the radicals that contain one or more unpaired electrons delocalized into the benzene ring. Different types of phenoxy radicals are present in nature like butylated hydroxytoluene (BHT), benzhydroxamic acid (BHA), flavonoids, etc. This paper primarily focuses on flavonoids. Flavonoids are a group of polyphenolic compounds found in fruits and vegetables. They act (in plants) as antioxidants, anti-microbials, photoreceptors, visual attractors, feeding repellants, and for light screening. They are benzo- $\gamma$ -pyrone derivatives, ubiquitous in vascular plants and are usually responsible for the bright colors found in plant tissues [5].

## Synthesis and structure of flavonoids

Flavonoids are formed from the combination of derivatives synthesized from phenylalanine (via the shikimic acid pathway) and acetic acid [1]. The first step involves the formation of phenylalanine from phenylpyruvate. Phenylalanine is transformed to trans-cinnamic acid, which is then hydrolyzed to *p*-coumaric acid (C-9). The C-9 acids condense with three C-2 (malonyl-coA) units to form a C-15 chalcone (Figure 1). Subsequent ring closure and hydration give rise to such compounds as the 3-hydroxyflavonoids (catechins) and 3,4-diolflavonoids (flavonols) [2].

The basic flavonoid structure consists of flavan nucleus, which has 15 carbon atoms arranged in three rings ( $C_6-C_3-C_6$ ) labeled A, B, and C [3]. The various classes of flavonoids differ in the level of oxidation and pattern of substitution of the C ring. The benzene ring A is condensed with a six-member ring (C), which in the 2-position carries a phenyl benzene ring (B) as a substituent (Figure 2). Ring C may be a heterocyclic pyran, which yields flavanols (catechins) and anthocyanidins, or pyrone, which yields flavonols, flavones, and flavanones [1].



**Figure 1.** The biosynthesis of the major classes of flavonoid derivatives. The enzymes are: CHS, chalcone synthase; CHR, chalcone reductase; CHI, chalcone isomerase; FSI, flavone synthase I; FSII, flavone synthase II; FLS, flavonol synthase; 'IFS', isoflavone synthase, consisting of 2-hydroxyisoflavanone synthase (2-HIS) and 2-hydroxyisoflavanone dehydratase (2-HID); F3bH, flavanone 3b hydroxylase; F39H, flavonoid 39-hydroxylase; F3959H, flavonoid 39,59-hydroxylase; DFR, dihydroflavonol reductase; ANS, anthocyanidin synthase; 3GT, anthocyanidin 3-glucosyltransferase; IOMT, isoflavone *O*-methyltransferase; IFR, isoflavone reductase; VR, vestitone reductase; DMID, 7,29-dihydroxy, 49-methoxyisoflavanol dehydratase. Enzymes in white are 2-oxoglutarate-dependent dioxygenases, in black bold are cytochrome P450s, and highlighted in gray are NADPH-dependent reductases. Simplifications include not discriminating between the 5-hydroxy (R1 5OH) and 5-deoxy (R15H) flavonoids and isoflavonoids, for which the loss of the 5-hydroxyl occurs because of the co-action of CHR with CHS, and showing only the anthocyanin pathway leading to the compounds with a di-substituted B-ring (cyanidin derivatives). Parallel pathways function in the formation of anthocyanins with mono- and tri-substituted B-rings. In the latter, F3959H can act at the level of the dihydroflavonol with a mono- or di-substituted B-ring. The pathway to epicatechin from a dihydroflavonol is shown to follow two routes, both via leucocyanidin [2]

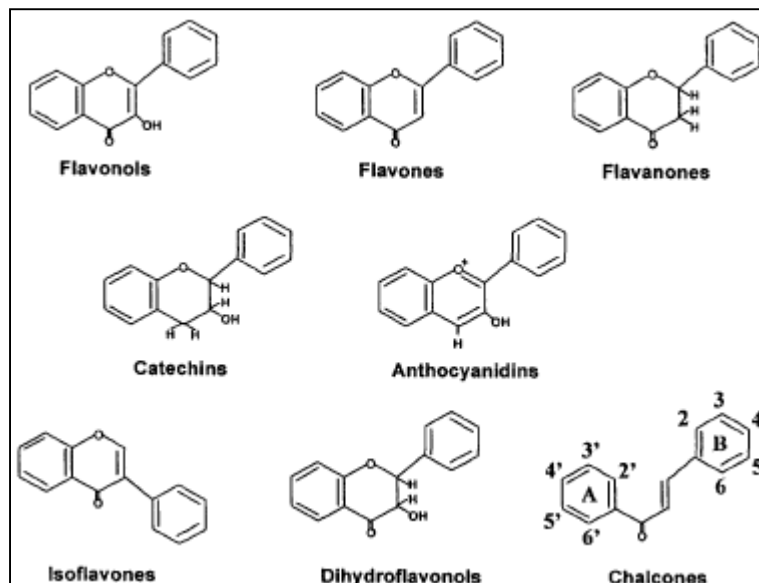


Figure 2: The main classes of flavonoids [4]

### Thermodynamic properties of flavonoids

The enthalpy and entropy of the reaction of superoxide with rutin (flavone) have been determined by the pulse conductivity at pH 10 and at 20°C by Jovanovic et al. (1994). The enthalpy ( $\Delta H$ ) is 3.6 kcal/mol and the entropy ( $\Delta S$ ) is  $-25$  cal/(mol K). Negative reaction entropy is the consequence of the solvent reorganization to accommodate the proton transfer in the transition state of electron transfer [9].

### Rate constants and transient spectra of flavonoids

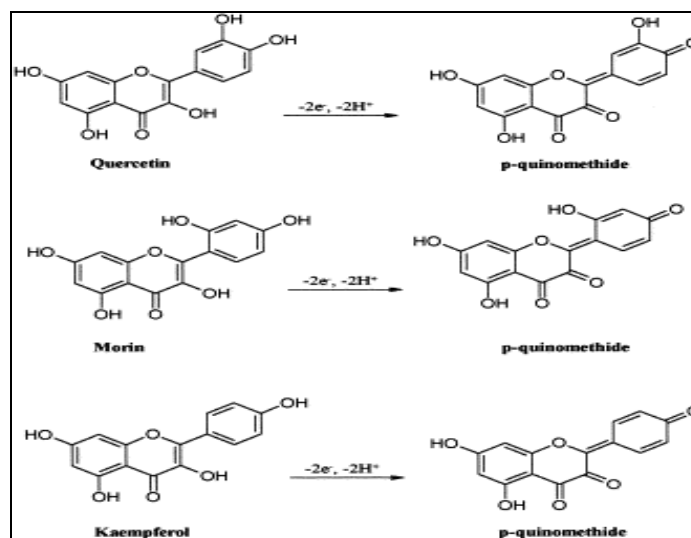
Rate constants for flavonoids are mostly obtained by pulse radiolysis, a method which observes fast absorption changes due to the formation and decay of intermediate radicals when the selective generation of individual radicals are combined with kinetic spectroscopy in the micro- and milli- second time range [6]. Cai et al [8] studied the interaction of hydrated electron ( $e_{aq}^-$ ) with flavonoids and determined the rate constants and the transient spectra for the different types of flavonoids which are given in table 1. The experiments were done at neutral pH (6.9) and room temperature. The rate constants of  $\bullet H$  reacting with flavonoids are approximately  $10^9 M^{-1} s^{-1}$  [8]. The rate constants of reaction of flavonoids with other species like  $\bullet OH$ ,  $\bullet N_3$  and  $t-BuO\bullet$  have also been reported which have been tabulated in table 1.

**Table 1:** Rate constants and transient spectra for the reactions of  $e_{aq}^-$  with flavonoids at pH 6.9 and room temperature ( $\pm$  standard deduction in five experiments) [7,8]

Substance	Rate Constant/ $M^{-1}s^{-1}$ (* $10^8$ )				(wavelength h) $\lambda_{max}/nm$	Transient spectra ( $\epsilon$ )/ $M^{-1}cm^{-1}$
	$\bullet OH$	$\bullet N3$	$t-BuO\bullet$	$e_{aq}^-$	$e_{aq}^-$	$e_{aq}^-$
Catechin (flavanol)	66	50	1.35	1.2 $\pm$ 0.1	< 320	>7* $10^3$
Epicatechin (flavanol)	64	51	-	-	-	-
Apigenin (flavones)	135	48	3.0	-	-	-
Luteolin (flavones)	130	41	5.7	-	-	-
Genistein (isoflavone)	-	-	-	62 $\pm$ 04)	<350	>2* $10^3$
					430	5* $10^2$
Genistin (isoflavone)	-	-	-	80 $\pm$ 10	<350	>2* $10^3$
					460	8* $10^2$
Rutin (flavone)	-	-	-	76 $\pm$ 4	<400	>2* $10^3$
Baicalin (flavanol)	-	-	-	110 $\pm$ 50	<400	>2* $10^3$
					460	1* $10^3$
Naringenin (flavanones)	210	52	2.65	120 $\pm$ 10	<370	>2* $10^3$
					480	1.5* $10^3$
Naringin (flavanones)	-	-	-	100 $\pm$ 10	<370	>2* $10^3$
					480	1.5* $10^3$
Quercetin (flavanol)	51	66	6.6	130 $\pm$ 50	<400	>2* $10^3$
					540	1* $10^3$

### Prooxidant toxicity

Same polyphenol compounds could behave as both antioxidants and prooxidants, depending on concentration and free radical source. Flavonoids autooxidize in aqueous medium, and may form highly reactive  $OH\bullet$  radicals in the presence of transition metals. Also, polyphenols and flavonoids may act as substrates for peroxidases and other metallo-enzymes, yielding quinone- or quinomethide-type prooxidant and alkylating product (Figure 3) [11].



**Figure 3:** Flavonoids as substrates for peroxidases yielding quinomethide [11]

### Flavonoids as Antioxidants

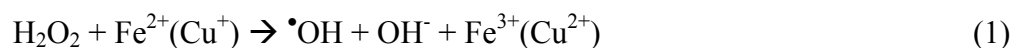
Mechanisms of antioxidant action include (1) suppressing ROS formation either by inhibition of enzymes or chelating trace elements involved in free radical production; (2) scavenging ROS; and (3) upregulating or protecting antioxidant defenses. Flavonoids fulfil most of the criteria required for the antioxidant action [10,12]. Thus the effect is two-fold:

1) Flavonoids inhibit the enzymes responsible for superoxide anion production, for instance xanthine oxidase and protein kinase C. Flavonoids have been also shown to inhibit the enzymes involved in the generation of ROS, such as cyclooxygenase, lipoxygenase, microsomal monooxygenase, glutathione S-transferase, mitochondrial succinoxidase, and NADH oxidase.

Flavonoids reduce the superoxide radicals in the pH range from 7 to 10, depending on their redox properties [9]. In addition to redox properties, the reactivity of superoxide also depends on the charge. For example, rate constant for reaction of superoxide with uncharged catechin at pH7 (determined by pulse radiolysis) is approximately 4 times higher than the corresponding rate at pH 10,  $k = 1.8 \times 10^4 \text{ M}^{-1} \text{ s}^{-1}$  where catechin is doubly negatively charged [9].



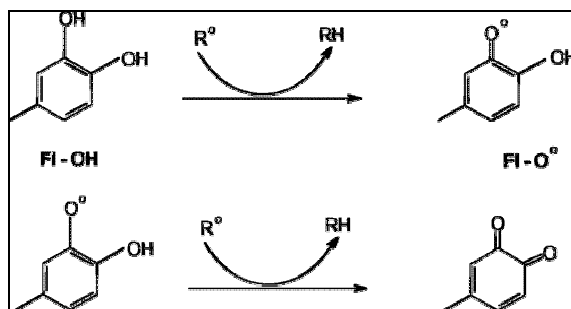
Furthermore, flavonoids efficiently chelate trace metals, which play an important role in oxygen metabolism (as shown in equation 1) [10]. Free iron and copper help in formation of reactive oxygen species (ROS), as exemplified by the reduction of hydrogen peroxide with generation of the highly aggressive hydroxyl radical. However, these metal ions are essential for many physiological functions, constituents of hemoproteins and cofactors of different enzymes, including those involved (e.g., iron for catalase, Cu for Cu,Zn-superoxide dismutase) in the antioxidant defense.



2) Due to their lower redox potentials ( $0.23 < E_7 < 0.75 \text{ V}$ ), same as compared to other anti-oxidizing species like trolox ( $E_7=0.48\text{V}$ ) and 4-methoxyphenol ( $E_7=0.73\text{V}$ ) [9], flavonoids (FI-OH) are thermodynamically able to reduce highly oxidizing free radicals with redox potentials in the range 0.13-1.0 V, such as superoxide radicals by hydrogen atom donation



Where,  $\text{R}^\bullet$  represents superoxide anion. The aroxyl radical (FI-O $^\bullet$ ) may react with a second radical, acquiring a stable quinone structure (Figure 4).



**Figure 4:** Scavenging of ROS by flavonoids [10]

The aroxyl radicals could interact with oxygen, generating quinones and superoxide anion, rather than terminating chain reactions. The last reaction may take place in the presence of high levels

of transient metal ions and is responsible for the undesired prooxidant effect of flavonoids. Thus, the overall capacity of flavonoids to act as antioxidants depends not only on the redox potential of the couple Fl-O<sup>•</sup>/Fl-OH but also on possible side reactions of the aroxy radical.

### **Roles in plant physiology**

Flavonoids play different roles in the physiology of plants. They may act as visual signals for pollinating insects due to their attractive colors. Because of their astringency, flavonoids can represent a defense system against insects harmful to the plant [10,12]. Flavonoids also act as catalysts in the light phase of photosynthesis and as regulators of ion channels involved in phosphorylation. They can also function as oxidative stress protectants in plant cells by scavenging ROS produced by the photosynthetic electron transport system. Moreover, flavonoids protect plants from solar ultraviolet (UV) radiation and scavenge UV-generated ROS, because of their favorable UV-absorbing properties. [10].

### **Summary**

Phenoxy radicals like polyphenols and flavonoids are common dietary components and have been studied over a period of time. The structural heterogeneity, wide occurrence, complex diversity, multiple mechanisms of action, and the diverse experimental methods used to evaluate their antioxidant activities have made flavonoids a very attractive system for chemical, genetic and enzymological studies. All flavonoids decay by second-order kinetics, *i.e.* bi-molecular disproportionation, forming quinones (quinone methides) and the parent hydroxy compound. Both antioxidant and prooxidant abilities of flavonoids originate from their reducing functions and thus largely depends on the hydroxylation in the backbone [8] and the overall capacity of flavonoids to act as antioxidants depends on the redox potential as well as on possible side reactions of the aroxy radical [10, 12].

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