Flavonoids and their free radical reactions

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Flavonoids - Introduction

- Flavonoids comprise a large group of secondary plant metabolites. Presently more than 5000 individual compounds are known, which are based on very few core structures. Their multitude derives mainly from the various hydroxylation patterns (up to six hydroxy groups) and ether substitution by simple methylation or diverse mono- and di-saccharides

  (Harborne JB, ed.: *The Flavonoids. Advances in Research*, Chapman & Hall, 1988)

- Their function in plants themselves most likely involves screening of UV light, *in situ* radical scavenging, anti-feeding effects (astringency), *etc*. Proanthocyanidins mostly occur in green tea (*Camellia sinensis*), grape seeds and skin (*Vitis vinifera*), or cacao (*Theobroma cacao*). The distinct occurrence of flavonoids makes them good candidates for taxonomic studies.

Flavonoids - Introduction

- Since the early fifties, their antioxidant potential with regard to food preservation has been a focus of research. Numerous papers exist on their inhibition of lipid peroxidation. Due to their structural variety, the flavonoids offer themselves also for detailed structure-activity relationship (SAR) studies.

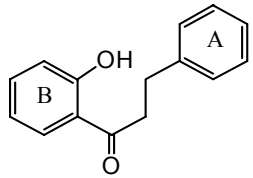
  (Bors W *et al.* (1990) *Meth. Enzymol*. **186**:343.)

- The antioxidant effect of flavonoids can reside both in their radical-scavenging activity or in their metal-chelating properties, of which the former may dominate.

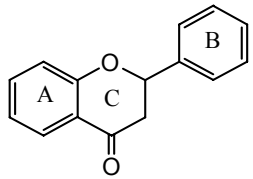
  (Bors W *et al.* (1996) in: *Handbook on Antioxidants*, Cadenas & Packer, eds, Dekker, New York, pp. 409.)

Core structures and nomenclature

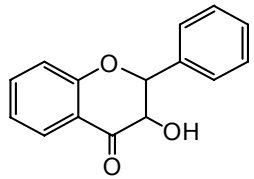
- The nomenclature of flavonoids proper is straight-forward with the aromatic ring A condensed to the heterocyclic ring C and the aromatic ring B most often attached at the C2 position. The various substituents are listed first for the A and C ring and - as primed numbers - for the B ring (note that the numbering for the aromatic rings of the open-chained precursor chalcones is reversed).
- (Harborne JB, ed. (1988) *The Flavonoids. Advances in Research*. Chapman & Hall.)



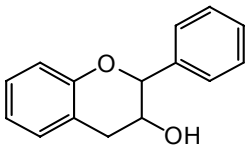
Chalcone



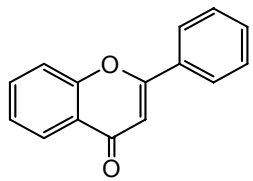
Flavanone



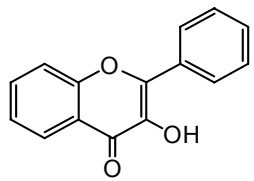
Dihydroflavonol



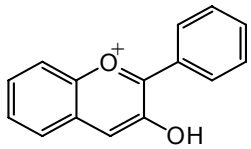
Flavan-3-ol



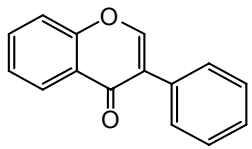
Flavone



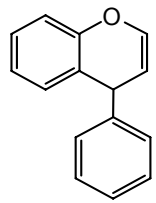
Flavon-3-ol



Anthocyanidin

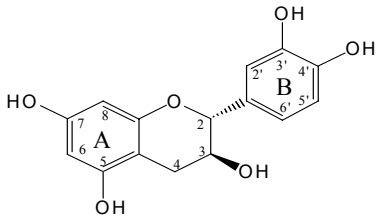


Isoflavone

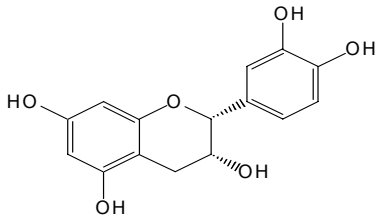


Neoflavone

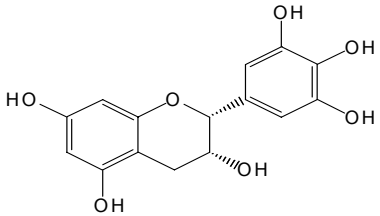
Flavan-3-ols



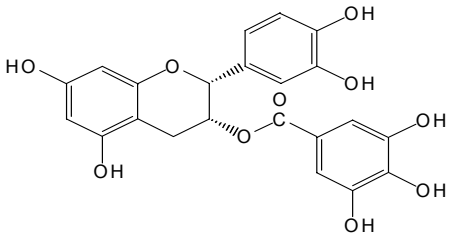
(+)-catechin (CAT)



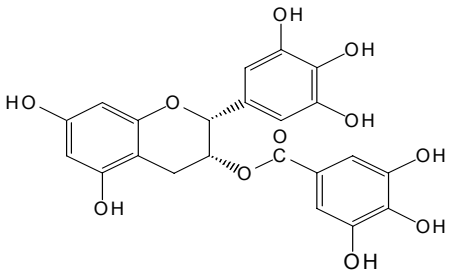
(-)-epicatechin (EC)



(-)-epigallocatechin (EGC)

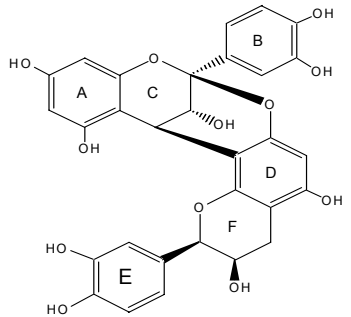


(-)-epicatechin gallate (ECG)

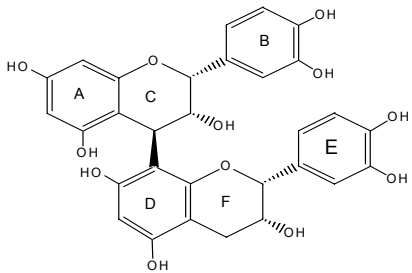


(-)-epigallocatechin gallate (EGCG)

Procyanidin dimers



procyanidin A2 (PC-A)



procyanidin B2 (PC-B)

The nomenclature for the flavan-3-ols is rather confusing, as it is based both on the actual structure, the chemical identification, and derivations thereof. For the proanthocyanidin oligomers, a highly systematic nomenclature exists, based on the structures of the monomers and the attachment sites. (Kaul (1996) *Pharmazie uns Zeit*. **25:**175.)

Radical scavenging - flavonoids

Scavenging rate constants of flavonoids for oxidizing radicals

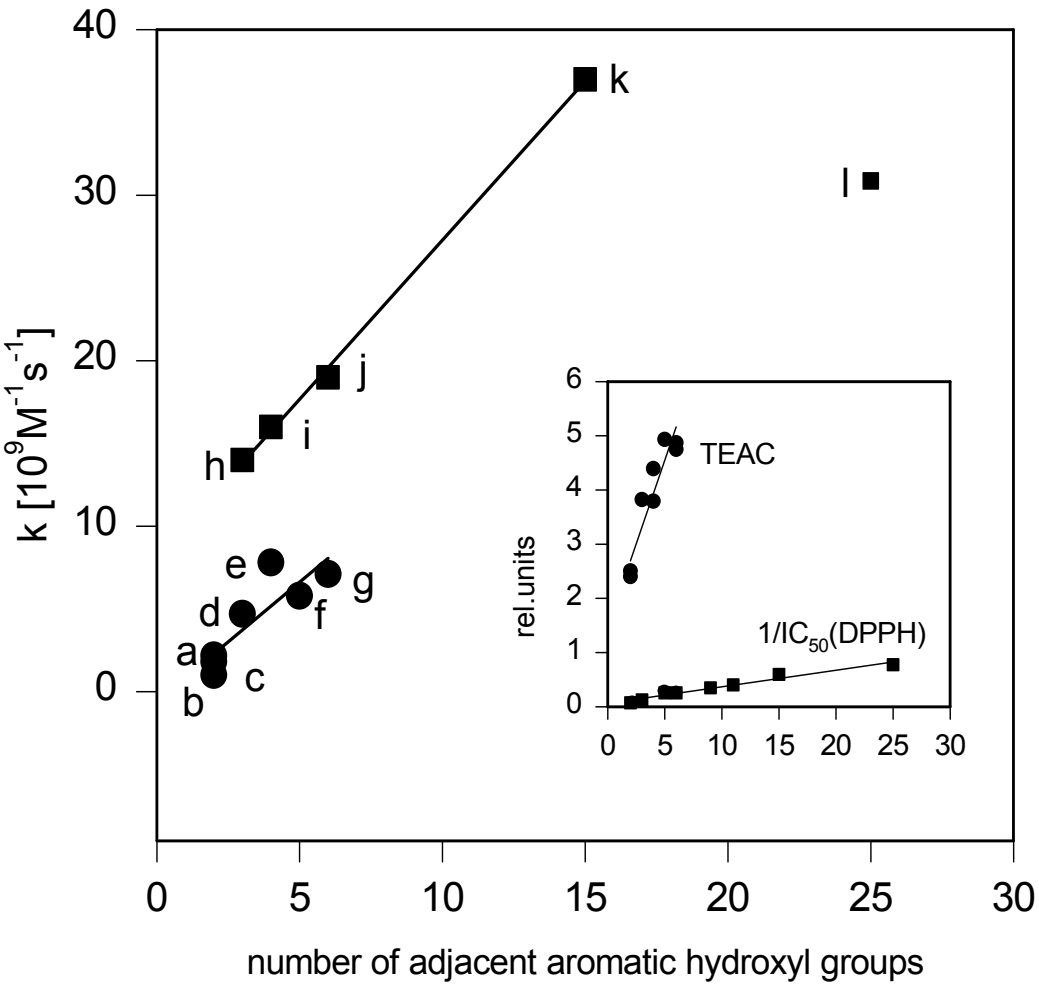
Substance (trivial name) (*substitution pattern*)	Rate constant ($x10^8$ $M^{-1}s^{-1}$) $^{\bullet}OH$	$^{\bullet}N_3$	*t*-BuO$^{\bullet}$
Flavanols			
(+)-catechin (*3,5,7,3',4'-penta-OH*)	66	50	1.35
(-)-epicatechin (- " -)	64	51	-
Flavanones, Dihydroflavonols			
naringenin (*5,7,4'-tri-OH*)	210	52	2.65
dihydrofisetin (*3,7,3',4'-tetra-OH*)	67	56	-
eriodictyol (*5,7,3',4'-tetra-OH*)	117	47	0.8
dihydrokaempferol (*3,5,7,4'-tetra-OH*)	58	89	0.95
dihydroquercetin (*3,5,7,3',4'-penta-OH*)	103	43	1.0
Flavylium salts (anthocyanidins)			
pelargonidine chloride (*3,5,7,4'-tetra-OH*)	45	62	-
Flavones			
apigenin (*5,7,4'-tri-OH*)	135	48	3.0
luteolin (*5,7,3',4'-tetra-OH*)	130	41	5.7
Flavonols (3-hydroxyflavones)			
kaempferol (*3,5,7,4'-tetra-OH*)	141	88	6.0
quercetin (*3,5,7,3',4'-penta-OH*)	51	66	6.6

The reaction of flavonoid aglycones with the electrophilic radicals $^{\bullet}OH$ or $^{\bullet}N_3$ are at the diffusion-controlled limits but within the same region for almost all investigated compounds. Except for rutin and hydroxyethylrutoside, very few studies with flavonoid glycosides exist - in fact, the antioxidant principle is based on the number and position of the various hydroxy groups. Other oxidizing radicals, *e.g.* *t*-BuO$^{\bullet}$, $O_2^{\bullet-}$, ROO$^{\bullet}$, *etc*. also react effectively with flavonoids, all forming the same transient aroxyl radicals. (Bors W *et al.* (1992) in: *Free Radicals and the Liver*, Csomos & Feher, eds, Springer, Berlin, pp. 77.)

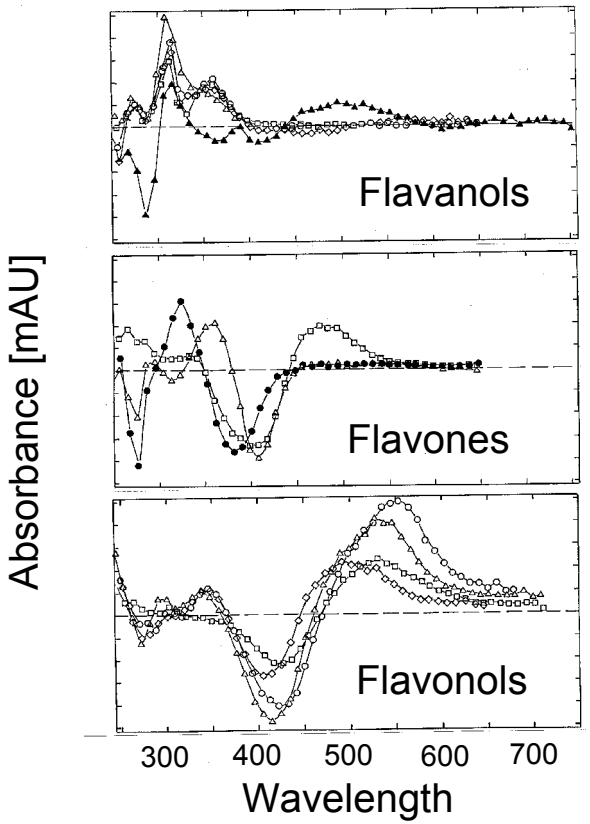
Radical scavenging - catechins

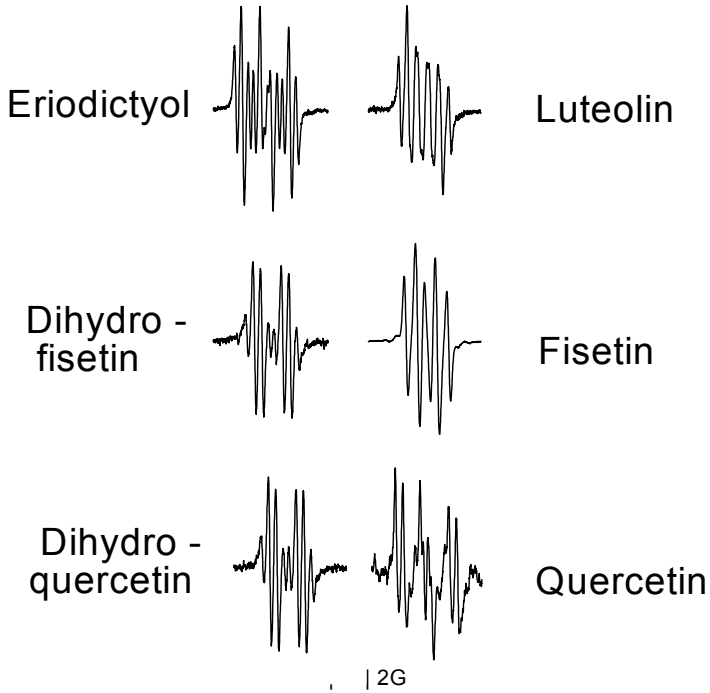
Only for the flavan-3-ols could a clear-cut SAR be established, in which case a linear increase of the rate constants with •OH correlate with the number of reactive hydroxy groups (*e.g.* the number of catechol or pyrogallol moieties). This correlation was not observed with $^{\bullet}N_3$ as it only reacts with dissociated phenols. Linear correlation is also seen in TEAC and DPPH assays.

(Bors W *et al*. (1999) *FRBM* **27:**1413.)
(Plumb *et al*. (1998) *Free Rad Res.* 29, 351.)
(Yokozawa *et al*. (1998) *Biochem Pharm.* **56:**213 .



Transient flavonoid radicals





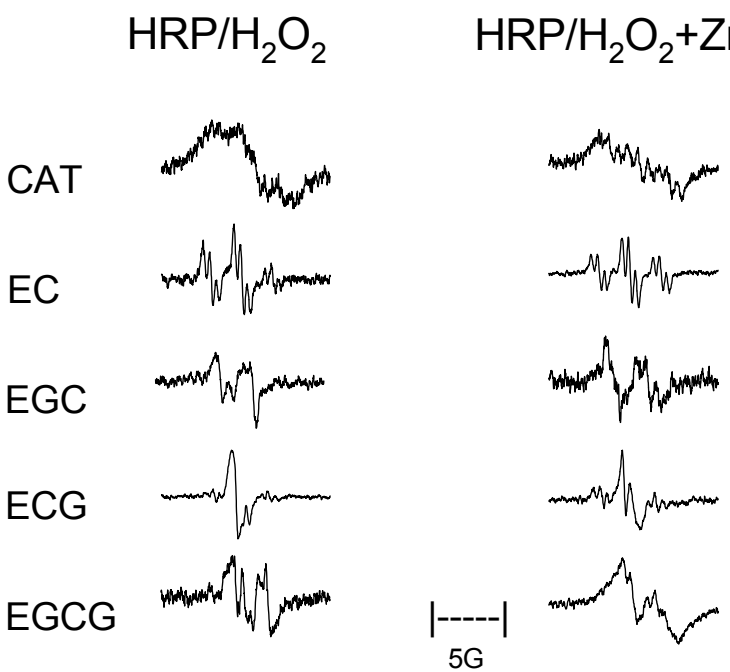
The transient spectra observed with the pulse-radiolytic technique reflect the B ring semi-quinones for all compounds with a saturated C ring (2,3-single bond). Desaturation leads to expanded delocalization of the odd electron over the whole three-ring system.

(Bors W *et al*. (1992) in: *Free Radicals and the Liver*, Cosmos & Feher, eds., Springer, Berlin, pp. 77.)

EPR spectra with HRP/H_2O_2 reveal the same structure of B ring semi-quinones. However, flavonol radicals (*e.g.* quercetin) are unstable and convert into other unresolved radicals structures.

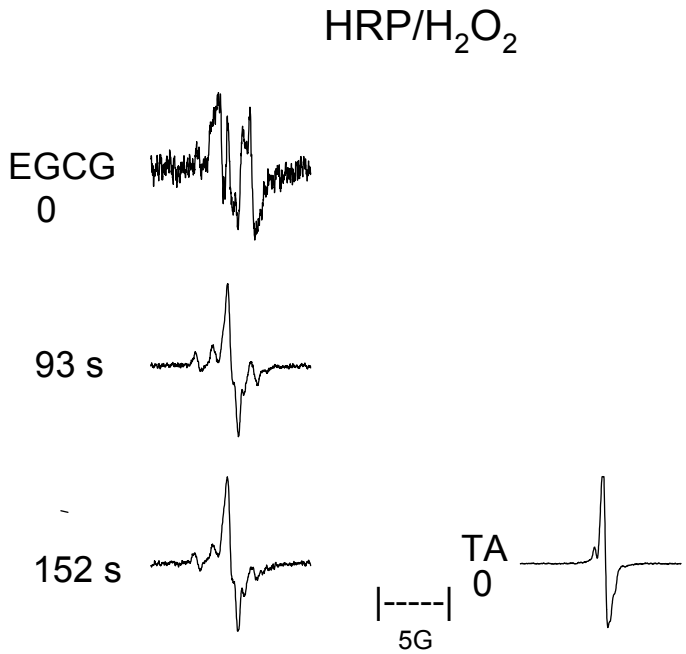
(Bors W *et al*. (1993) in: *Free Radicals*, Poli *et al*., eds., Birkhäuser, pp. 374.)

Transient flavonoid radicals



EPR spectra of flavan-3-ols have also been studied using the 'spin stabilization' technique. (Kalayanaraman, *Meth. Enzymol*. **186**:333, 1990)

The results show the limitation of that method, as it seems not to improve the signal of the pyrogallol semiquinones and only enhances the catechol semiquinone signal of epicatechin gallate (ECG). (Bors W *et al*. (2000) *Arch Biochem Biophys*. **374**:347.)



For catechins, the propensity for oligo-merization is reflected in the EPR spectra as well, leading from well-resolved signals to single line signals of polymers.
(Bors W *et al*. (2000) *Arch Biochem Biophys.* **374**:347.)

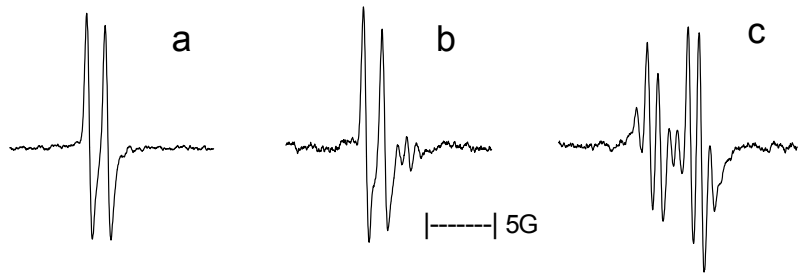
Reaction scheme of interactions between flavonoids and ascorbate after initiation of radical reactions with azide radicals

[0]	$^{\bullet}OH + N_3^-$	→	$^{\bullet}N_3 + OH^-$
[1]	FlOH	↔	$FlO^- + H^+$
[2]	$^{\bullet}N_3 + FlO^-$	→	$FlO^{\bullet} + N_3^-$
[3]	$^{\bullet}N_3 + AscH^-$	→	$Asc^{\bullet-} + N_3^-$
[4]	$2\ ^{\bullet}N_3$	→	$3\ N_2$
[5a,b]	$FlO^- + Asc^{\bullet-} + H^+$	↔	$FlO^{\bullet} + AscH^-$
[6]	$2\ FlO^{\bullet}$	→	FlOH + Fl=O
[7a,b]	$Fl=O + Asc^{\bullet-} + H^+$	↔	$FlO^{\bullet}$ + DHA
[8]	$FlO^{\bullet} + Asc^{\bullet-}$	→	FlO^- + DHA
[9]	$FlO^{\bullet} + Asc^{\bullet-}$	→	$Fl=O + AscH^-$
[10]	$2\ Asc^{\bullet-} + H^+$	→	$AscH^-$ + DHA

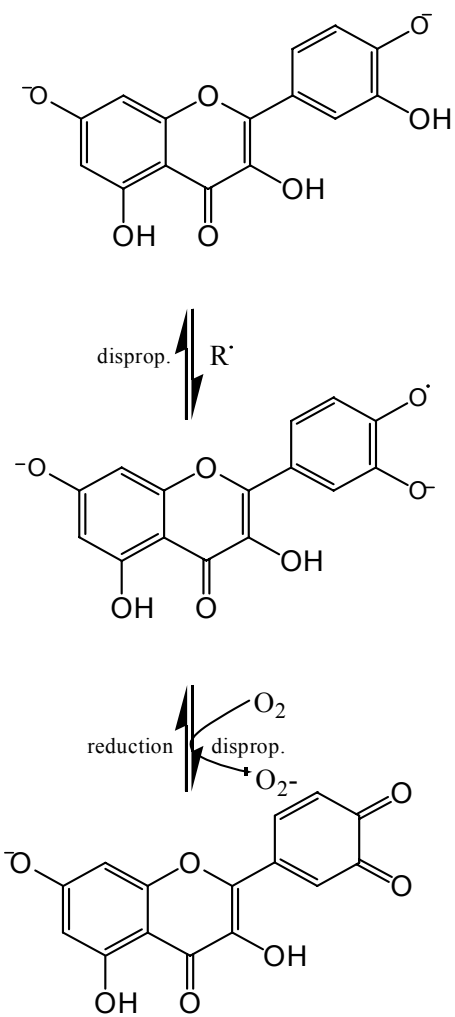
$FlOH/FlO^-$, $FlO^{\bullet}$, Fl=O denote the dissociation and univalent oxidation steps of the flavonoids; $AscH^-$, $Asc^{\bullet-}$ and DHA those of ascorbate; reactions [5a,b] and [7a,b] represent the two univalent redox equilibria, reactions [8] and [9] are two radical/radical elimination reactions, the direction determined by the respective reduction potentials.

As antioxidants, flavonoids are by definition capable of electron-transfer reactions. Among those, the reaction with ascorbate has been studied in detail, with kinetic modeling of the complex scheme allowing the determination of the respective redox potentials. (Bors W *et al*. (1995) *FRBM* **19:**45.)

Redox properties



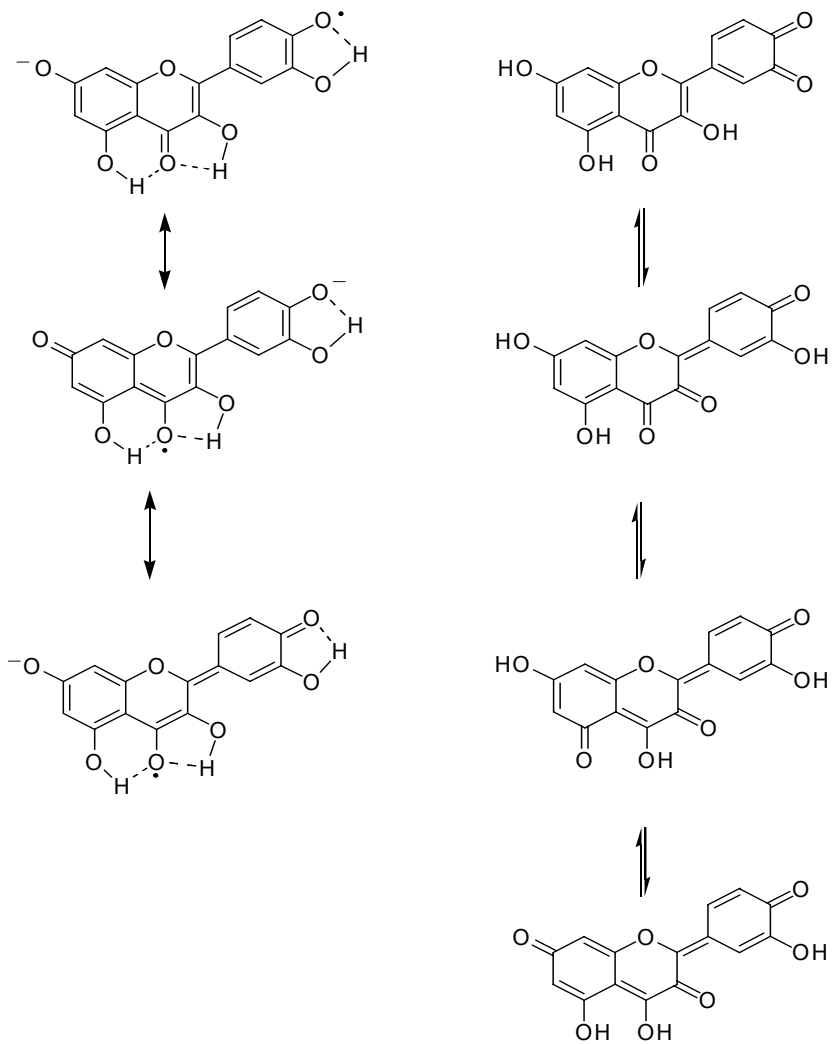
In the case of dihydroquercetin (c), whose optical semiquinone spectrum is superimposed over that of ascorbate (a) and its radical, EPR spectra describe the reducing power of dihydroquercetin *vis-a-vis* the ascorbate radical (note that the signal intensity of the latter radical is much higher).
(Bors W *et al*. (1997) *J Magnet Reson Anal.* **3**:149.)



Mechanism of radical interactions - flavonols

Formation of flavonoid aroxyl radicals is an essential step after initial scavenging of an oxidizing radical. The stability of the aroxyl radicals strongly depends on their bimolecular disproportionation reaction and electron delocalization. Furthermore, the quinones formed from the resp. semiquinones behave quite distinctly from the flavonoids proper and the flavan-3-ols: in the first case, quinones can undergo (futile) redox cycling, with $O_2^{\bullet -}$ formed in a pro-oxidant effect. (Metodiewa *et al.* (1999) *FRBM* **26:**107.)

Mesomeric structures of quercetin radicals and quinone methides



Mechanism of radical interactions - flavonols

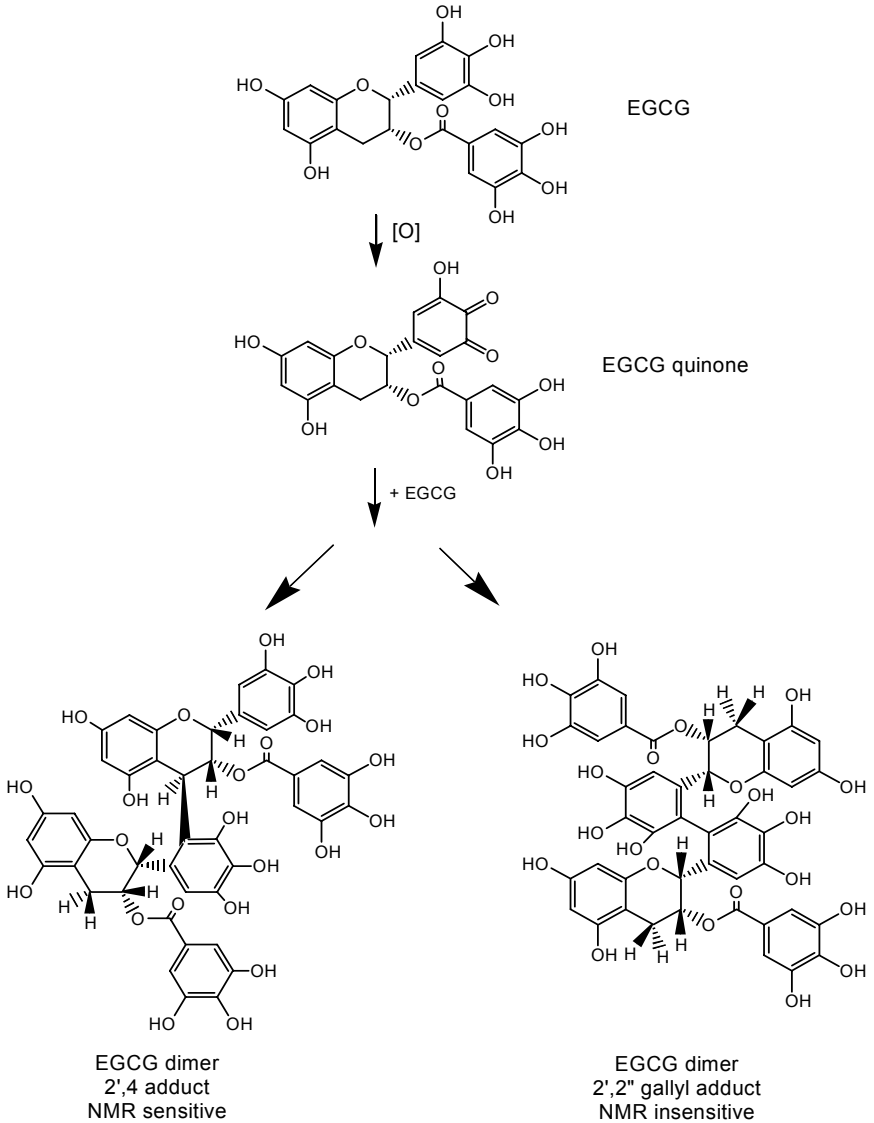
The quinone methides, *e.g*. those of flavonols are prone to nucleophilic attack, which with DNA might lead to pro-mutagenic adducts.
(Boersma *et al.* (2000) *Chem Res Toxicol* **13:**185;

Awad *et al.* (2001) *Chem Res Toxicol*. **14:**398.)

Mechanism of radical interactions - catechins

In contrast to the potentially pro-oxidant flavonoid quinones, those of catechins (flavan-3-ols) preferentially react *via* phenolic coupling reactions (S_N2) to form dimers and oligomers, each retaining its original number of reactive hydroxy groups, *i.e.* enhancing its antioxidant capacity until a level is reached when the oligomers become insoluble and precipitate.

(Bors W *et al.* (2001) *Antiox Redox Signal.* **3:**995.)



Summary and Conclusions

- Flavonoids in general scavenge oxidizing (electrophilic) radicals preferentially *via* their B-ring catechol or pyrogallol units at diffusion-controlled rates.
- In proanthocyanidins, due to oligomerization, several catechol/ pyrogallol sites exist for radical attack.
- All flavonoid aroxyl (semiquinone) radicals decay by second-order kinetics, *i.e.* bi-molecular disproportionation, forming quinones (quinone methides) and the parent hydroxy compound.
- Quinones and quinone methides of flavonols especially may have pro-oxidant activities due to either futile redox cycling or nucleophilic attack.
- Quinones of proanthocyanidins preferentially dimerize *via* phenolic coupling reactions, thereby enhancing the antioxidant potential with each oligomerization step.