JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Kinetic and Stoichiometric Assessment of the Antioxidant Activity of Flavonoids by Electron Spin Resonance Spectroscopy

DONALD B. MCPHAIL,*,† RICHARD C. HARTLEY,‡ PETER T. GARDNER,† AND GARRY G. DUTHIE†

Rowett Research Institute, Greenburn Road, Bucksburn, Aberdeen AB21 9SB, Scotland, and Department of Chemistry, University of Glasgow, University Avenue, Glasgow G12 8QQ, Scotland

There is current interest in the use of naturally occurring flavonoids as antioxidants for the preservation of foods and the prevention of diseases such as atherosclerosis and cancers. To establish the molecular characteristics required for maximum antioxidant activity, electron spin resonance (ESR) spectroscopy has been used to determine the stoichiometry and kinetics of the hydrogen-donating ability of 15 flavonoids and D-α-tocopherol to galvinoxyl, a resonance-stabilized, sterically protected aryloxyl radical. The second-order reaction rates, which will be governed by O−H bond dissociation energies, were myricetin > morin > quercetin > fisetin ∼ catechin > kaempferol ∼ luteolin > rutin > D-α-tocopherol > taxifolin > tamarixetin > myricetin 3′,4′,5′-trimethyl ether > datiscetin > galangin > hesperitin ∼ apigenin. Reactivity is highly dependant on the configuration of OH groups on the flavonoid B and C rings, there being little contribution from the A ring to antioxidant effectiveness. Highest reaction rates and stoichiometries were observed with flavonols capable of being oxidized to orthoquinones or extended paraquinones. However, rates and stoichiometries did not always correlate and the data suggest that kinetic factors may be of greater importance within a biological context.

KEYWORDS: ESR; flavonoids; antioxidant capacity; kinetics; stoichiometry; galvinoxyl radical

INTRODUCTION

Flavonoids (**Figure 1**) are a group of more than 4000 naturally occurring polyhydroxyphenolic products of the phenylpropanoid biosynthetic pathway in plants (*1*). They are present in a wide range of fruits, vegetables, nuts, and beverages including wine and tea (*2*−*5*). The flavonoids exhibit diverse in vitro biological activities of pharmacological significance including enzyme regulation, the modulation of ecosanoid synthesis, promotion of xenobiotic metabolism, and the suppression of tumor growth (*6*). Most recent attention has been directed to the antioxidant properties of flavonoids because their extensive conjugated π-electron systems allow ready donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals (*7*). In addition to being potential alternatives to the commonly used synthetic phenolics for the food industry (*8*), they may have health benefits because epidemiological studies indicate that adequate intakes of flavonoid-rich foods may decrease the risk of coronary heart disease and certain cancers (*9*). These benefits may occur, in part, as a result of inhibition of the oxidation of low-density lipoprotein to an atherogenic form and by minimizing oxidative damage to DNA (*10*). Stoichiometric studies, comparing the reducing capabilities of flavonoids to Trolox (a vitamin E analogue) in aqueous systems, indicate that this antioxidant ability is markedly influenced by the position of hydroxyl groups on the B and A rings and the extent of conjugation between the B and C rings (*11*). However, results from hydrophilic media may not be directly comparable to antioxidant effectiveness in lipid-rich foods (*12*). Moreover, within a biological system where a number of polyphenols may be present at similar concentrations, antioxidant efficacy may be predominantly governed by reaction kinetics rather than stoichiometry. Consequently, this study has assessed the antioxidant potential of 15 flavonoids and vitamin E and compared their kinetic and stoichiometric reduction of a synthetic radical using stopped-flow electron spin resonance (ESR) spectroscopy. The radical used was galvinoxyl (Galv-O•) [2,6-di-*tert*-butyl-α-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-*p*-tolyloxy], which is resonance-stabilized and sterically protected and

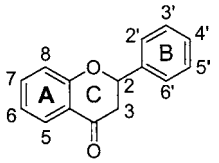

Figure 1. Basic ring structure of flavonoids with labeling convention.

* To whom correspondence should be addressed (telephone +44-(0)-1224-712751; fax +44-(0)1224-715349; e-mail d.mcphail@rri.sari.ac.uk).
† Rowett Research Institute.
‡ University of Glasgow.

10.1021/jf025922v CCC: $25.00
Published on Web 02/07/2003

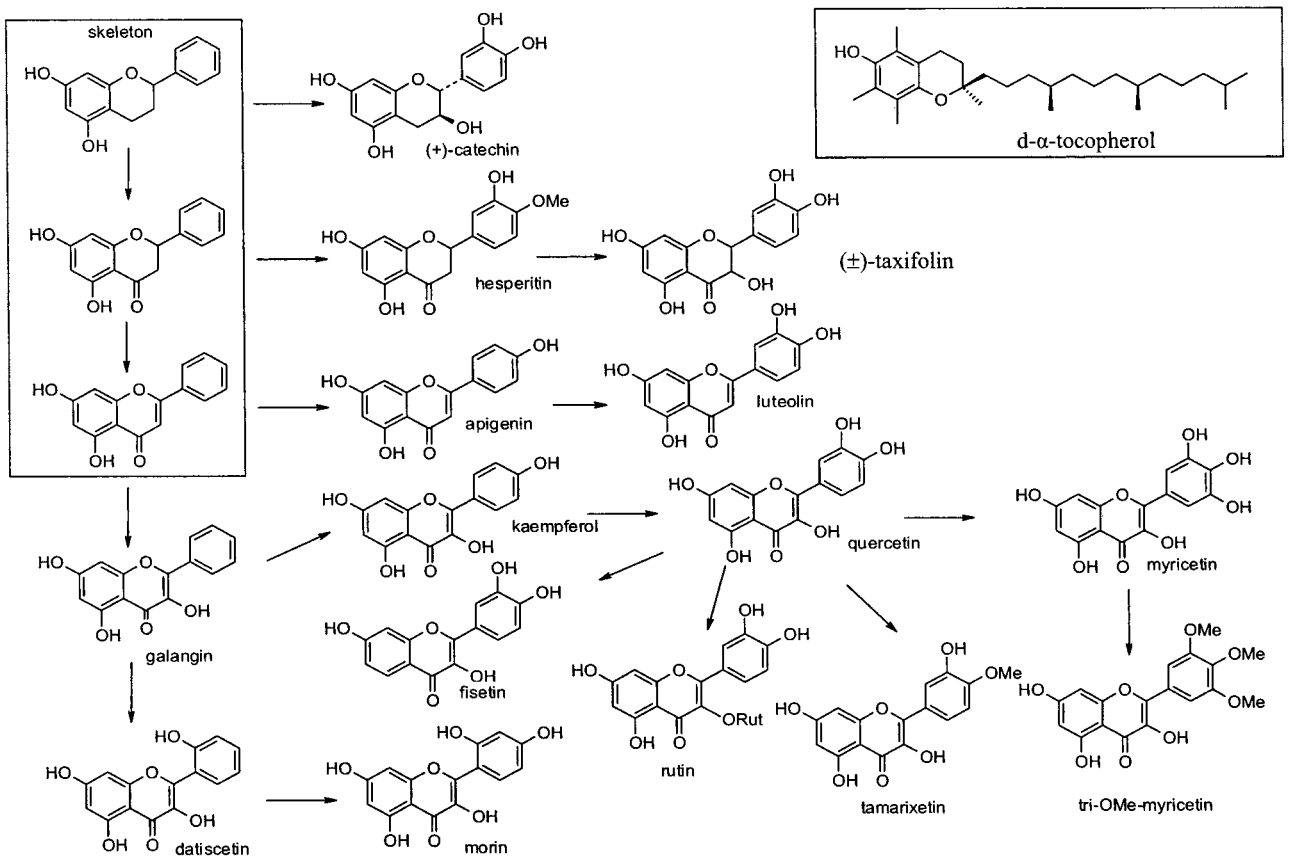

Figure 2. Relationship of flavonoids to the basic ring systems and to each other by modification of substitution patterns. The structure of D-α-tocopherol (vitamin E) is in the inset for comparison.

so displays little self-reactivity in solution and is reduced by H-atom transfer reactions in the presence of phenolic compounds,

$$\text{Galv-O}^{\bullet} + \text{phenol-OH} \leftrightarrow \text{Galv-OH} + \text{phenol-O}^{\bullet}$$

the process being governed by the O−H bond dissociation enthalpy of the donor group (*13*). Galvinoxyl has a well-defined ESR spectrum, and we have used this property to calculate second-order rate constants, as well as establishing stoichiometry, for the reaction with phenolic compounds.

MATERIALS AND METHODS

Materials. Tamarixetin and myricetin 3′,4′,5′-trimethyl ether (**Figure 2**) were purchased from Indofine Chemical Co. (Somerville, NJ). The remaining flavonoids (**Figure 2**), D-α-tocopherol and galvinoxyl [2,6-di-*tert*-butyl-α-(3,5-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylidene)-*p*-tolyloxy] were purchased from Sigma-Aldrich Chemical Co. (Poole, Dorset, U.K.) and ethanol (> 99.7%) was from BDH Laboratory Supplies (Poole, Dorset, U.K.). Reagents were used without further purification.

Kinetic Measurements. Ethanolic solutions of flavonoid (0.2 mM) and galvinoxyl (0.2 mM) were deoxygenated under a stream of nitrogen gas. Aliquots (6 mL) were transferred to Hamilton gastight syringes (10 mL) coupled to a pneumatic ram and connected to a two-stream ESR quartz flow cell. In situ reaction at 20 ± 2 °C between the flavonoid and galvinoxyl was initiated by rapidly evacuating the syringes. Spectra and decay curves were obtained on a Bruker ECS 106 spectrometer operating at ca. 9.5 GHz (X band) and equipped with a TM_{110} cavity. Decay curves were obtained by operating in time sweep mode with the static field set at the resonance maximum of the galvinoxyl signal. To enhance stability, the signal was modulation-broadened using an amplitude of 0.127 mT. Conversion times of 84 or 21 ms were used as appropriate for the signal decay rates with respective time constants of 5.12 and 2.56 ms. A microwave power of 1.01 mW was employed throughout. Three decay curves were obtained for each flavonoid, the stopped-flow system being purged with deoxygenated ethanol between experiments. The time sweep spectrum of a galvinoxyl/ethanol control was acquired for calibration. Comparison between experimental and control spectra ensured that the flow rate through the cell was sufficiently high such that >95% of the galvinoxyl was unreacted at the stop of flow. Second-order rate constants (k_2) were calculated from the ESR decay curves using the integrated rate expression applicable where initial reactant concentrations are equivalent (*14*); i.e.,

$$k_2 t = \frac{b}{a(a-b)} \quad (1)$$

where t = time after start of reaction, a = initial concentration of galvinoxyl, and b = number of moles of galvinoxyl reacted after time t. Ten data points were sampled from each decay curve within the first 50% of the reaction (i.e., where 0.05 mM < [galvinoxyl] < 0.1 mM), and k_2 was obtained by plotting t against $b/[a(a-b)]$. The mean and standard deviation were calculated for three injection replicates, and correlation coefficients were obtained to indicate how well the data fit the second-order model.

Stoichiometric Measurements. Ethanolic solutions of flavonoids (0.1 mM) were prepared. Aliquots (3 mL) of an ethanolic galvinoxyl solution (0.5 mM) were mixed with an equal volume of flavonoid solution and then transferred to an ESR quartz cell. Evaluation of spectra and reaction stoichiometry were as previously described for catechin derivatives (*15*). In brief, the spectra of the unreacted galvinoxyl were obtained 5 min from mixing, by which time equilibration was complete. The galvinoxyl concentrations remaining were calculated by double integration of the signal and comparing with the control experiment where ethanol was added to the galvinoxyl solution instead of to the flavonoid solution.

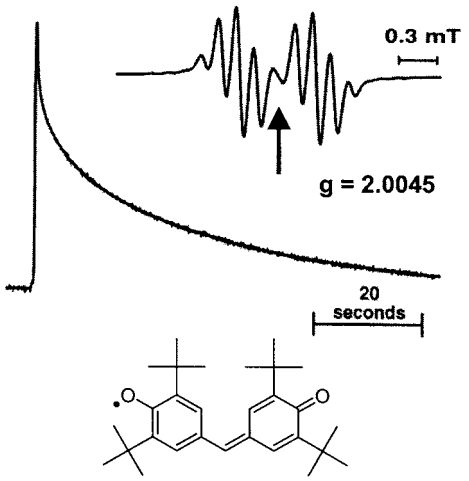

Figure 3. Decay curve of the galvinoxyl resonance obtained in ESR time sweep mode (static field) during in situ reduction of the radical by quercetin. Inset is the radical structure and its field sweep spectrum.

RESULTS

The ESR spectrum of galvinoxyl in an ethanolic solution consists of a doublet of quintets (**Figure 3**) that arise from the interaction of the unpaired electron spin with the nuclear spins of the proton on the central carbon and the four equivalent aromatic ring protons. In the presence of a hydrogen-donating compound such as quercetin, the resonances decay as reduction of the radical proceeds (**Figure 3**). Data from all the decay curves gave a good linear fit to the second-order integrated rate expression, with the average correlation coefficient for each set of replicates being greater than 0.970. However, there were marked differences between the flavonoids in the kinetics of the reduction of the galvinoxyl free radical (**Table 1**). Myricetin and morin were, by far, the fastest to react, whereas hesperitin and apigenin showed little reactivity. Ranking of reaction rates as second-order rate constants was myricetin > morin > quercetin > fisetin ~ catechin > kaempferol ~ luteolin > rutin > taxifolin > tamarixetin > myricetin 3′,4′,5′-trimethyl ether > datiscetin > galangin > hesperitin ~ apigenin. Reaction rates of eight of the flavonoids were greater than that for vitamin E.

The stoichiometry of the reaction of these compounds with the galvinoxyl free radical was determined by adding the flavonoid, or vitamin E, to an excess of the radical and allowing the reaction to proceed to the endpoint. This resulted in a ranking of antioxidant capacity that differed from the kinetic ranking (**Table 1**) i.e., myricetin > fisetin > quercetin ~ luteolin > rutin > catechin > taxifolin > kaempferol ~ morin > datiscetin > tamarixetin > myricetin 3′,4′,5′-trimethyl ether ~ galangin > hesperitin > apigenin. In particular, the reaction of morin with galvinoxyl had the second fastest rate of all the compounds but was only ranked eighth equal in terms of the number of radicals reduced. Seven of the flavonoids had a greater reaction stoichiometry than vitamin E. Datiscetin, galangin, hesperitin, and apigenin were the four lowest ranked of all the compounds in both the kinetic and stoichiometric measurements of antioxidant potential.

DISCUSSION

A large number of natural phenolic compounds in fruit, vegetables, tea, and wines have antioxidant activity due to their hydrogen donor activity and their ability to complex transition metal ions (*12*, *18*). In addition to the location and total number of hydroxyl groups, the solubility of the phenolics in the test medium may significantly affect their ability to act as antioxidants (*19*). For example, antioxidant activity of flavonoids in lard appears to be related to the number of *o*-dihydroxy grouping in the A and B rings (**Figure 1**) (*12*) whereas a lack of conjugation between the B and C rings is a major influence in aqueous media (*11*).

The first two reactions between quercetin and the galvinoxyl radical (**Figure 4**) show that abstraction of two hydrogen atoms leads to the formation of tautomeric orthoquinone **B** and extended paraquinone **C**. All flavonoids that can form quinone or extended quinones by loss of two hydrogen atoms have a stoichiometry of greater than 1.7, while those that cannot form such compounds have a stoichiometry of 1.1 or less. Stoichiometries of greater than 2 result from further hydrogen abstraction following solvent addition to the quinone or extended quinone moiety (*20*).

In the first reaction with the galvinoxyl radical, a hydrogen atom is abstracted from the 4′-OH of quercetin to give a semiquinone radical stabilized by hydrogen bonding from the 3′-OH and by conjugation with both the 3-OH and the 3′-OH. It is the rate constant for this step, k_2, that we have determined. Since the 4′-OH bond is cleaved homolytically in this step, k_2 will depend on the bond dissociation energy (BDE) of this phenolic hydroxyl, i.e., the difference between the energy of the phenoxyl radical **A** (and abstracted hydrogen atom), described as the radical energy RE, and the ground-state energy of the phenol, quercetin, GE. Since the hydrogen atom is transferred to the same radical (the galvinoxyl radical) in all experiments, the relative rates of reaction can be discussed in terms of factors that affect the BDE of the phenolic hydroxyls of the flavonoids studied. In each case, stabilization of the phenol (e.g., quercetin) increases the BDE and so decreases reaction rate, while stabilization of the phenoxyl radical (e.g., **A**) lowers the BDE and so increases reaction rate. The oxygen atom of the reacting phenolic hydroxyl is a strong π donor, while the oxygen atom of a phenoxyl radical is a strong π acceptor. Consequently, electron-withdrawing groups, such as carbonyl groups, that are in direct conjugation with this oxygen atom slow reaction by lowering GE and raising RE, increasing the BDE. Conversely, electron-donating groups, such as hydroxyl groups, that are in direct conjugation with this oxygen atom accelerate reaction by lowering RE and raising GE, decreasing the BDE. In the case of quercetin, the 3-OH and 3′-OH decrease the BDE of the 4′-OH while the carbonyl group increases this BDE.

Attempts to quantify the way in which the nature and position of substituents on the aromatic ring affect BDEs of phenolic hydroxyls have used ΔBDE, where the ΔBDE for a substituent X is calculated as shown in eq 2. ΔBDEs for a variety of substituents have been calculated and determined experimentally (*21*−*29*). The calculated and experimental values are generally in good agreement, and the contribution of each substituent to the BDE of polysubstituted phenols appears to be additive. Unfortunately, computations tend to underestimate the donating effects of hydroxyl groups (*28*), particularly in polar solvents where they act as hydrogen-bond donors and so have increased electron density on the oxygen atom (*23*). Matters are further complicated when intramolecular hydrogen bonding of the phenoxyl radical is possible as in semiquinone **A**. Such hydrogen bonding stabilizes the radical and decreases the BDE, but the effect is decreased by solvents that are good hydrogen-bond acceptors so that ΔBDE is smaller in these solvents (*25*).

$$\Delta\text{BDE} = (\text{BDE of } C_6H_5OH) - (\text{BDE of } XC_6H_4OH) \quad (2)$$

The absolute value of the BDE for a given phenol varies with solvent, and reaction rates are generally lower in solvents that

Table 1. Second-Order Rate Constants (k_2) and Reaction Stoichiometries for the Reduction of Galvinoxyl Radical by Flavonoids and Vitamin E[a]

compound	k_2 (mol^{-1} dm^3 s^{-1})	reaction stoichiometry	substitution pattern							
			3	4	5	7	2′	3′	4′	5′
catechin[b]	1574 ± 79	2.96 ± 0.01	–H, –OH	–H, –H	–OH	–OH		–OH	–OH	
taxifolin	337 ± 32	2.82 ± 0.05	–H, –OH	=O	–OH	–OH		–OH	–OH	
hesperitin[b]	6 ± 0.5	0.20 ± 0.02	–H, –H	=O	–OH	–OH		–OH	–OMe	
apigenin[b]	5 ± 0.5	0.04 ± 0.02	–H	=O	–OH	–OH			–OH	
luteolin[b]	1212 ± 45	3.24 ± 0.01	–H	=O	–OH	–OH		–OH	–OH	
galangin	18 ± 1	1.01 ± 0.03	–OH	=O	–OH	–OH				
fisetin	1623 ± 199	3.68 ± 0.03	–OH	=O		–OH		–OH	–OH	
kaempferol[b]	1243 ± 99	1.84 ± 0.01	–OH	=O	–OH	–OH			–OH	
quercetin[b]	2383 ± 258	3.27 ± 0.04	–OH	=O	–OH	–OH		–OH	–OH	
tamarixetin	164 ± 20	1.14 ± 0.03	–OH	=O	–OH	–OH		–OH	–OMe	
rutin[b]	670 ± 41	3.18 ± 0.01	–ORut	=O	–OH	–OH		–OH	–OH	
myricetin[b]	14463 ± 1767	4.08 ± 0.01	–OH	=O	–OH	–OH		–OH	–OH	–OH
tri-OMe-myricetin	74 ± 14	1.06 ± 0.02	–OH	=O	–OH	–OH		–OMe	–OMe	–OMe
datiscetin	22 ± 2	1.74 ± 0.02	–OH	=O	–OH	–OH	–OH			
morin	10134 ± 459	1.83 ± 0.01	–OH	=O	–OH	–OH	–OH		–OH	
vitamin E	524 ± 48	2.14 ± 0.12								

[a] Rutin is quercetin-3-rutinoside. Catechin, taxifolin, and hesperitin are based on the 2-H flavan system, while the rest of the compounds are based on Δ-2-flavan-4-ones. [b] Significant dietary-derived flavonoids (*16*, *17*).

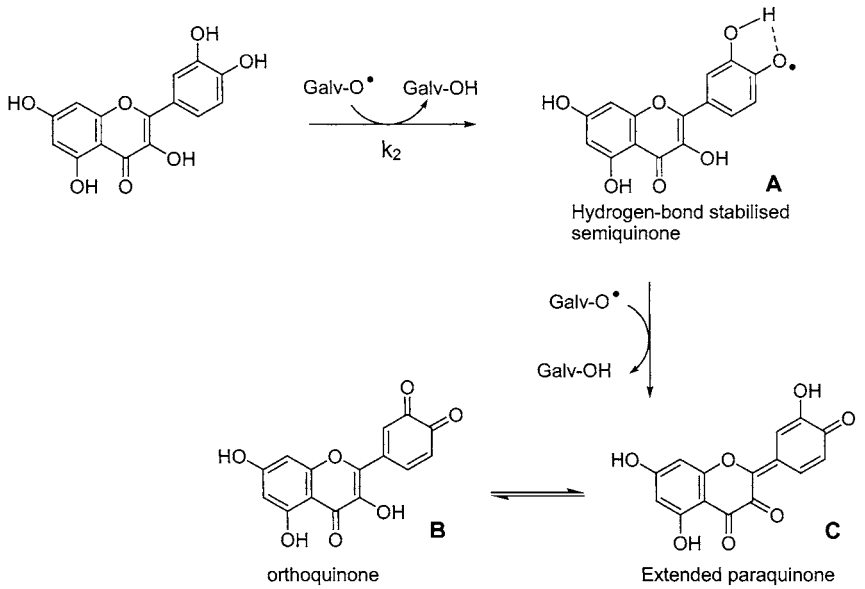

Figure 4. Two-stage oxidation of quercetin by galvinoxyl radical to its quinonoidal forms via the semiquinone radical intermediate.

are good hydrogen-bond acceptors (*30*−*33*). Although ΔBDE for many substituents is unaffected by solvent, this is not true for *o*-hydroxyls, and quercetin has been reported to react with oxygen-centered radicals 8 times more slowly than α-tocopherol in chlorobenzene (which is not a hydrogen-bond acceptor) but 30 times more slowly in *tert*-butyl alcohol (which is a hydrogen-bond acceptor and was considered by the authors to be a "water-like" solvent) (*30*). Significantly, our kinetic data show that quercetin and seven other flavonoids react more quickly than α-tocopherol in ethanol. Ethanol is considerably more "water-like" than *tert*-butyl alcohol, being a better hydrogen-bond acceptor because it is more polar [the dielectric constants (*34*) of *tert*-butyl alcohol, ethanol. and water are 10.9 (30 °C), 24.3 (25 °C), and 78.54 (30 °C), respectively] and its hydroxyl group is less sterically hindered.

When flavonoids are considered, there are two further complications to interpreting kinetic results. First, coplanarity of the B and C rings may be sterically disfavored, and this will affect conjugation between them. Quercetin is essentially planar when crystalline with a C(2)−C(1′) torsion angle of only 1°, but this torsion angle is much larger when there is a substituent at C(2′) (*35*). However, information from crystal structures has to be interpreted with caution because the C(2)−C(1′) torsion angle can vary by as much as 60° when the same flavone is crystallized in different environments (*36*). Indeed, the barrier to rotation about the C(2)−C(1′) bond of myricetin, which lacks a C-2′ substituent, has been calculated to be less than 2 kcal mol^{-1} (*36*). When there is a C(2)−C(3) single bond, the relative orientation of the B and C rings is less important, and the C ring may vary from being coplanar with the B ring as in the crystal structure of hesperitin to being perpendicular as in that of naringenin (*37*). Second, the BDEs of a number of phenolic hydroxyls may be close in energy, and the kinetic data are then a composite of a number of different reactions. These difficulties mean that only a qualitative explanation of our data is possible at this stage.

Galangin reacts 4 times more quickly than apigenin, indicating that the intrinsic reactivity of the 3-OH is higher than that of the 4′-OH. This is perhaps due to conjugation from the lone pair of the oxygen atom at position 1 to the 3-O• of the oxyl radical derived from galangin.

Luteolin reacts 242 times more quickly than apigenin because the semiquinone is stabilized by intramolecular hydrogen

bonding, so the BDE of the parent phenol is lower (*25*). An *o*-methoxyl group has very little effect on BDE because it is incapable of forming such a bond. This is clear from the 56-fold increase in rate on going from hesperitin to taxifolin. Catechin and luteolin react at similar rates, indicating that the electronic contribution from the C ring in each compound is similar. The lower rate for reaction of taxifolin relative to catechin may be a consequence of the carbonyl group withdrawing electron density from the oxygen atom at position 1 of taxifolin by conjugation through the A ring. This in turn will lead to greater polarization of the O(1)−C(2) bond so that the C ring will decrease electron-donation/increase electron-withdrawal from the B ring.

Kaempferol reacts 249 times more quickly than apigenin because the 3-OH lowers the BDE of 4′-OH and vice versa. Thus, addition of either a 3-OH or a 3′-OH to apigenin gives rise to a similar rate enhancement. Quercetin combines these factors and reacts 477 times more quickly than apigenin. This is only a 2-fold increase on the reaction rates of luteolin and kaempferol, so the electronic factors do not appear to be additive. Presumably, the 4′-OH is reacting preferentially in quercetin because only a 4′-O$^{\bullet}$ would be stabilized by conjugation with two hydroxyl groups. The 15-fold decrease in reactivity when the 4-OH is methylated, as in tamarixetin, is consistent with this. Tamarixetin reacts more quickly than galangin, as would be expected from its more electron-rich B ring. Quercetin reacts with an oxygen-centered radical 1.5 times more quickly than catechin in ethanol, and this agrees well with the factor of 1.24 observed for quercetin and epicatechin in *tert*-butyl alcohol (*30*).

Myricetin has a second *o*-hydroxyl group and so reacts 6 times more quickly than quercetin. Only 4′-O$^{\bullet}$ will benefit from conjugation with three hydroxyl groups, and it will be most easily formed. The slow reaction rate for trimethylated myricetin is consistent with this. Preferential hydrogen abstraction from the 4-OH of 3,4,5-trihydroxybenzoate (gallate) esters has already been demonstrated (*25*). Surprisingly both trimethylated myricetin and tamarixetin react more slowly than kaempferol. This implies that either the 4′-OH of kaempferol is more reactive than the 3-OH or that a 4′-methoxyl group has considerably less effect than a 4′-hydroxyl group on the BDE of the 3-OH. However, the former explanation seems to be at odds with the relative reactivities of galangin and apigenin.

The most intriguing results are those of morin and datiscetin that have a 2′-OH. Datiscetin reacts at essentially the same rate as galangin that lacks the 2′-OH. The reaction stoichiometry however is higher and close to that of kaempferol, implying that an extended orthoquinone is formed. Morin, on the other hand has a reaction rate 563 times greater than galangin and a stoichiometry almost identical to that of kaempferol. The 2′-OH sterically disfavors the B and C rings being coplanar in datiscetin, particularly in a polar solvent where a hydrogen bond between the 2′-OH and the oxygen atom at position 1 is unlikely. Thus, the 2′-OH will have little effect on the BDE of the 3-OH. However, coplanarity of the B and C rings is more favorable in morin because both the 2′-OH and 4′-OH would be directly conjugated with the carbonyl group. Semiempirical AM1 calculations show that the barrier to rotation about the C(2)−C(1′) bond of morin is about 28 kcal mol^{-1} because eclipsing of the 2′-OH and 3-OH is disfavored but that coplanarity with an antirelationship is only disfavored by 3 kcal mol^{-1} (*36*). Both the 2′-OH and 4′-OH of morin will contribute to the lowering of the BDE of the 3-OH, favoring formation of 3-O$^{\bullet}$. Formation of 4′-O$^{\bullet}$ from morin will also be more favorable than formation of the corresponding 4′-O$^{\bullet}$ from kaempferol because the 2′-OH is in direct conjugation with the electron-withdrawing carbonyl group, and this will negate the latter's effect on the BDE of the 4′-OH. Both factors will contribute to the high reactivity of morin. The pK_a of morin is reported as 3.46, which is uncharacteristically low for a flavonol (generally these have a pK_a of 6.8−8.2) (*38*). If this figure is correct, then morin will be partially ionized in the unbuffered ethanol solution and the phenoxide ion will react much more quickly than the parent flavonol (the reaction rates for the reaction of flavonoid anions with superoxide radicals at pH10 have been reported (*38*)).

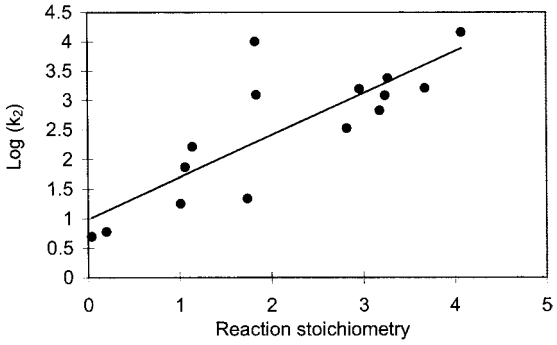

Figure 5. Correlation between log of the second-order rate constants (k_2) and the stoichiometry of the reaction between galvinoxyl radical and 15 flavonoids.

The reaction rate for quercetin is 3.6 times greater than its 3-glycosylated analogue, rutin. The 3-OH may contribute to the overall rate of galvinoxyl consumption by quercetin and this is absent in rutin. Furthermore, the bulky rutinosyl group disfavors the trigonal geometry that would allow optimum overlap of the lone pair of the C(3) oxygen atom with the C(2)−C(3) double bond. Thus, the C(3) oxygen atom will not stabilize the 4-O$^{\bullet}$ of the phenoxy radical. The orientation of the rutinosyl group in the crystal structure of rutin supports this argument (*39*). Rutin still reacts 134 times more quickly than apigenin because it is capable of forming a hydrogen-bond-stabilized semiquinone.

Of the 15 flavonoids examined, 8 had rate constants greater than that of vitamin E. The antioxidant activity of vitamin E, from a kinetic perspective, has previously been ascribed to the positive inductive (+I) effect of the three methyl groups on the aromatic ring and the resonance stabilization (+M) contributed from the heterocyclic oxygen in which the stereochemistry of the heterocyclic ring enforces good overlap of the oxygen p-orbital electron pair with the aromatic π-system (*40*). The increased reactivity of the flavonoids with respect to vitamin E may result from three factors: (1) a more extended conjugated system to support an unpaired electron, (2) two or more OH groups capable of a +M contribution, and (3) less steric hindrance at the site of abstraction.

A correlation ($r = 0.818$) was found between log(k_2) and the stoichiometric parameters for the reduction of galvinoxyl by flavonoids (**Figure 5**). However, not all compounds followed this trend. In particular, datiscetin, kaempferol, and morin had almost identical reaction stoichiometries (ca. 1.8), yet the reaction rates were 22, 1243, and 10134 mol^{-1} dm^3 s^{-1}, respectively. These results highlight the importance of considering reaction kinetics, as well as stoichiometry, when assessing antioxidant capacity. Where two, or more, potential antioxidants are present, as may occur in complex cellular environments, kinetic factors may greatly override reaction stoichiometry in determining which compound will afford the greatest protection.

CONCLUSION

Flavonoids, such as quercetin, may get absorbed from the diet into tissues (*41*). Consequently, kinetics and stoichiometry must both be considered in assessing the relevance of plant phenolics as nutritional antioxidants for disease prevention. This ESR method is a useful model to determine these two distinct aspects of antioxidant activity for lipophilic compounds. The galvinoxyl radical is insufficiently oxidizing to indiscriminately abstract H atoms from a wide range of substrates. Therefore, reactions are only likely to be significant with good H donors, i.e., compounds that may fulfill an antioxidant role within a biological context. For a particular class of compound, the method has considerable potential in identifying groups involved in H-atom transfer and the effect of ring substituents in altering reactivity through inductive, resonance, and steric mechanisms. We have shown that eight flavonols react more quickly than α-tocopherol with an oxygen-centered radical in a water-like solvent (ethanol). To have high reaction rates and high reaction stoichiometries, the flavonols must be capable of being oxidized to orthoquinones or extended paraquinones. Higher reaction rates and stoichiometries are obtained for quercetin, which is capable of forming either an orthoquinone or an extended paraquinone, but the effects of the 3-OH and 3′-OH on the BDE of the 4′-OH do not appear to be additive. The relationship between rates and reaction stoichiometries is not a simple one. Myricetin, which has a 5′-OH in addition to the 3-OH, 3′-OH, and 4′-OH groups of quercetin, has the highest reaction rate and reaction stoichiometry, but while it reacts much more quickly than quercetin, its reaction stoichiometry is only slightly greater. More dramatically, morin, which has a 2′-OH in addition to the 3-OH and 4′-OH of kaempferol, reacts 8 times more quickly than kaempferol but has the same reaction stoichiometry. Consequently, kinetic factors may be of overriding importance in determining protection within a biological context.

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Received for review August 28, 2002. Revised manuscript received December 23, 2002. Accepted December 30, 2002. This study was funded by the EC (Grant FAIR-CT95) and the Scottish Executive Environment and Rural Affairs Department (SEERAD).

JF025922V