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Experimental Determination of Octanol−Water Partition Coefficients of Quercetin and Related Flavonoids

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Octanol−water partition coefficient (log *P*) values were determined for flavonoids from the flavone, flavonol, flavanone, and isoflavonoid subclasses. Each flavonoid was dissolved in an octanol−water system and allowed to equilibrate, and then both fractions were analyzed by high-performance liquid chromatography. log *P* was calculated as log[ratio of the concentration in the octanol phase to the concentration in the aqueous phase at pH 7.4]. The aglycons were more lipophilic than any conjugate. The conjugate moiety had a more significant effect on log *P* than the aglycon moiety. Quercetin was the least lipophilic aglycon (log $P = 1.82 \pm 0.32$) and, together with kaempferol (log $P = 3.11 \pm 0.54$), gave the most variable results. The isoflavones genistein and daidzein and the isoflavone metabolite equol gave relatively high log *P* values (3.04 ± 0.02, 2.51 ± 0.06, and 3.20 ± 0.13, respectively), while glycitein had an unexpectedly low value of 1.97 ± 0.05. The conjugation characteristics and hydroxylation pattern were the most important determinants of log *P* in general, and log *P* was highly variable within the flavonoid subclass. The results are discussed in terms of further understanding of the in vivo fate of the flavonoids as important dietary bioactives.

KEYWORDS: Partition coefficients; log *P*; flavonoids; isoflavones; quercetin; dietary nonnutrients; phytochemicals

INTRODUCTION

Flavonoids comprise over 4000 compounds owing to huge diversity in the configuration and substituents of their phenolic rings. Flavonoids are found in almost all plants and are therefore consumed in significant amounts by humans. Onions, for example, are rich in glycosides of the flavonol quercetin (*1*), which is a strong antioxidant (*2*) and whose intake has been inversely correlated with plasma LDL concentration (*3*). The consumption of flavonoids has been associated with protection against coronary heart disease (*4*, *5*) and cancer (*6*). Isoflavone consumption has been associated with relief from menopausal symptoms (*7*, *8*). However, human absorption and metabolism studies have been constrained by inadequate analytical techniques, the difficulty of in vivo studies (particularly given poorly understood inter- and intraindividual variations), and a lack of understanding of the interactions of flavonoids with other dietary components.

In vivo fate is fundamentally dependent upon lipophilicity and hydrophilicity, which govern membrane and protein interactions. The importance of lipophilicity in vivo is such that it can be expressed as a partition coefficient, a parameter that relates the manner in which a single solute partitions between polar and nonpolar phases (*9*). Partition coefficient determinations were originally performed using oil and water. It was later realized that using an octanol nonpolar phase better simulated the environment within a living tissue, because, like membrane lipids, 1-octanol has a hydrophobic, long alkyl chain and a polar OH group (*10*). As such, the octanol−water partition coefficient (log *P*) best represented the behavior of a compound toward a membrane. log *P* is defined as the logarithm of the ratio of the solute concentration in the octanol phase to the solute concentration in the water phase, at a defined temperature.

Few studies have been devoted to an understanding of log *P* values for flavonoids. Values in the literature are scattered, having usually been used as a secondary or peripheral part of a larger study, e.g., Murota et al (*11*). Consequently, finding log *P* data by searching the literature is unrewarding. log *P* determination appears straightforward, but accurate, precise, and reproducible experimental data are scarce, since the methodology is time-consuming and fraught with difficulties. Modeled data are more commonly encountered. Fujita et al. (*12*) were the first to develop a calculation method for log *P*; many other publications since have treated the calculation of log *P* from the chemical structure, most using fragment addition with correction factors taking into account fragment interaction (*9*, *13*, *14*). Experimentally, the most direct and probably most effective method is to dissolve the analyte in an octanol−water system, mix the two phases vigorously, separate them, and quantify the analyte in one or both fractions.

The aim of this study was to determine log *P* accurately for a range of flavonoids, and to observe how log *P* relates to what

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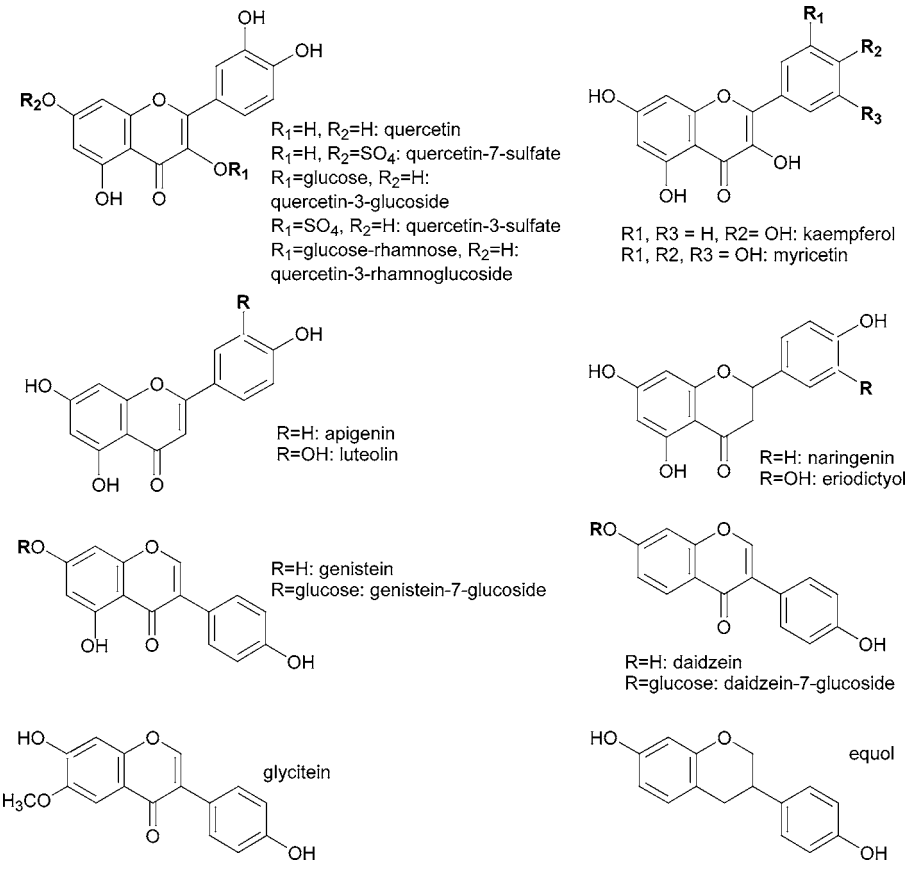

Figure 1. Structures of the flavonoids used in the study.

is known about their absorption and metabolism. Flavonoids studied were from the flavone, flavonol, flavanone, and isoflavone subclasses (**Figure 1**). Since levels of aglycons are comparatively low in food plants relative to glycosylated forms, various conjugates were studied. The data were compared with existing experimental and modeled data.

MATERIALS AND METHODS

Naringenin and quercetin were obtained from Sigma Chemical Co., Dorset, U.K. Apigenin, daidzein, equol, eriodictyol, genistein, glycitein, luteolin, kaempferol, myricetin, quercetin-3-glucoside (isoquercitrin), quercetin-3-rhamnoglucoside (rutin), quercetin-3-sulfate, daidzein-7-glucoside (daidzin), and genistein-7-glucoside (genistin) were obtained from Extrasynthese, Genay, France. Quercetin-7-sulfate was synthesized and donated by Denis Barron of the University of Lyon, France (*15*).

The methodology was based on that of Murota et al. (*11*). Solutions of each flavonoid were prepared in methanol (Fisher Chemicals, Loughborough, U.K.) filtered previously through 0.2 μm filter units (Chromos, Macclesfield, U.K.) and aliquots placed in 2 mL Eppendorf vials (100 μL). Solutions of 0.2−1.0 mM were used, depending on the sensitivity of the detector response for each compound at the chosen wavelength. The methanol was evaporated either using a stream of nitrogen or by air-drying, leaving a residue of 100 nm. Filtered 1-octanol (200 μL, Sigma) or Tris−HCl buffer (200 μL, pH 7.4) was added to the flavonoid residue. The residues were dissolved by vigorous mixing using a whirlimixer (Fisherbrand, Loughborough, U.K.) for 1 min. If afterward crystals were still visible, the solutions were incubated in a water bath at 40 °C for at least 30 min or until they had dissolved. The remaining phase was then added and each replicate mixed for a further 1 min. The two phases were centrifuged using an Eppendorf 5415C desktop centrifuge (5 min, 2900*g*). If precipitation occurred, centrifugation was substituted by unaided separation for 30 min. Following separation, most of the octanol fraction was removed for HPLC analysis. The remainder of the octanol was carefully removed and discarded and an aliquot of the buffer fraction removed for analysis. log *P* was calculated by taking the logarithm of the ratio of the HPLC peak area of each compound in octanol to the corresponding peak area in buffer. Consequently, log *P* values were based on analysis of both aqueous and octanol fractions.

The average recovery was determined for each set of replicates as a check of reliability and consistency, though under the described experimental protocol recovery does not impact directly the determination of the log *P* value. An aliquot of the methanol solution from which each flavonoid residue was derived was analyzed by HPLC. Recovery was calculated as the ratio of the total quantity of flavonoid detected in the buffer and octanol phases to the quantity of flavonoid in the equivalent amount of stock solution.

Reversed-phase HPLC was used to analyze aqueous and octanol fractions. The system used was a Merck-Hitachi Lachrom L-series with an L-7200 autosampler and a D-7000 quaternary pump coupled to an L-7450 diode array detector. The column used was a C18 packed with Prodigy ODS3 silica, of 250 mm length by 4.6 mm internal diameter (Phenomenex, Macclesfield, U.K.). Solvent A was Milli-Q-purified water containing trifluoroacetic acid (0.1%, Sigma), and solvent B was acetonitrile (Fisher Chemicals, HPLC grade). The solvents were pumped through the system at a flow rate of 1 mL/min. A 35 min gradient was used, starting at 17% solvent B, increasing to 25% at 7 min, 35% at 50%, 50% at 20 min, and finally 90% at 25−28 min, preceding a 6 min postrun (17% B). Injection volumes were 40 μL for octanol and buffer fractions and 10 μL for recovery standards. Eluent was monitored between 220 and 400 nm using diode array detection. Peaks were integrated at a suitable wavelength for each compound. The identity of each compound was confirmed by the retention time and spectroscopic properties.

RESULTS

log *P* data are presented in **Table 1**. The most lipophilic flavonoid was luteolin (log $P = 3.22$), giving a slightly higher value than the isoflavone metabolite equol. The most hydrophilic was quercetin-3-sulfate (log $P = -1.11$). The conjugates had considerably lower log *P* values than the aglycons, independent

Table 1. Experimentally Determined log *P* Values and HPLC Retention Times

flavonoid	log *P* ± SD (*n* = 5)	retention time in MeOH/min
luteolin	3.22 ± 0.08	20.08
equol	3.20 ± 0.13	23.15
kaempferol	3.11 ± 0.54	23.72
genistein	3.04 ± 0.02	22.70
apigenin	2.92 ± 0.06	22.90
naringenin	2.60 ± 0.03	22.30
daidzein	2.51 ± 0.06	18.35
eriodictyol	2.27 ± 0.02	20.13
glycitein	1.97 ± 0.05	18.28
quercetin	1.82 ± 0.32	20.50
genistein-7-glucoside	0.97 ± 0.01	12.56
quercetin-3-glucoside	0.76 ± 0.01	12.21
quercetin-7-sulfate	0.74 ± 0.02	14.72
daidzein-7-glucoside	0.32 ± 0.02	8.76
quercetin-3-rhamnoglucoside	–0.64 ± 0.05	10.88
quercetin-3-sulfate	–1.11 ± 0.01	11.23
myricetin	a	15.64

[a] Value not determined due to instability in the aqueous phase.

of the subclass. The log *P* of quercetin-3-sulfate (−1.11) was significantly lower than that of quercetin-7-sulfate (0.74), and glycitein aglycon had a lower log *P* (1.97) than its nonmethoxylated counterpart daidzein (2.51) and a much lower log *P* than genistein (3.04). Coefficients of variation were both small in value and consistent across the range of compounds tested. Recovery, in the majority of cases, was in excess of 90% and consistent between replicates (data not shown). log *P* data did not always correspond to the relative mobilities of the flavonoids on the reversed-phase column, as shown in **Table 1**.

Quercetin had the lowest log *P* (1.82) of any aglycon tested. Quercetin and kaempferol partitioned into the octanol−buffer system only with considerable difficulty, and their replicates produced coefficients of variation of ±0.32 and ±0.54, respectively, compared to ≤±0.13 for all other compounds.

log *P* could not be determined for myricetin. HPLC analysis of the aqueous phase after partitioning revealed that the flavonoid degraded during the procedure, as evidenced by the presence of additional, earlier eluting peaks that were not present in the standard. These observations were made on a consistent basis, regardless of whether the flavonoid was dissolved first in the octanol or the aqueous phase.

DISCUSSION

Partitioning processes between phases occur frequently in vivo, and therefore, log *P* not only is an indicator of the affinity of a compound for a membrane, but also has many other uses. log *P* determination has become the standard approach for predicting the transport and binding activity of xenobiotics. When a drug is in development, many different candidates are initially screened. A predetermined range of log *P* (indicating the probable potential for absorption) might be used to eliminate those which would be unlikely to be readily absorbed from the gut. log *P* is also a crucial indicator in studies of the environmental fate of organic chemicals since it can be correlated with various geochemical parameters. It even has regulatory significance in Japan, where data are required for each new agrochemical or pharmaceutical compound manufactured or imported (*13*). log *P* is related to other physical parameters inherent in the molecule such as water solubility. This can be predicted from log *P* (*14*). log *P* is strongly implicated in the study of intermolecular forces between organic compounds, and it is possible to relate partitioning-like processes by means of simple equations (*16*). For instance, the log *P* of a small organic molecule can be related theoretically to its binding affinity for serum albumin (*17*), the magnitude of which influences tissue distribution and excretion.

The physicochemical properties of flavonoids determine their in vivo characteristics of absorption and distribution. To elicit biological effects in vivo, dietary flavonoids must be absorbed and distributed to tissues via systemic circulation. In the absence of active transport systems, the bioactive must diffuse passively across biological membranes, not only for absorption from the gut, but also to enter cells and tissues from systemic circulation. Only molecules of appropriate lipophilicity can diffuse across phospholipid membrane. Those too hydrophilic are unavailable to the cell interior, and those too hydrophobic unable to react in aqueous conditions. The log *P* of a compound is also inherently related to the bioactivity itself. For example, the ability of a flavonoid to inhibit lipid peroxidation is diminished if too lipophilic or hydrophilic (*18*).

The aglycons of luteolin, a flavone from celery and sweet peppers, and equol, a mammalian metabolite of daidzein, were the most lipophilic (log *P* = 3.22 and 3.20, respectively). The sulfate, glucoside, and rhamnoglucoside conjugates had the lowest log *P* values, at least one unit lower than that of any aglycon, apart from quercetin. The intramolecular forces which give rise to hydrophilicity bestowed by the addition of a sugar moiety are much more influential on log *P* than those created by OH groups substituted on the rings, and even aglycons bearing numerous hydroxyl groups may have high log *P* values. log *P* for a glucose substituent is known to be about −2.4 (*19*) compared to −0.27 for a hydroxyl group (estimated using the Molinspiration on-line interactive model). The different structures of the subclasses exerted a lesser effect on log *P* than that produced by addition of a conjugate moiety. There is no obvious pattern of distribution of log *P* within subclasses, and values were closer between flavonoids which have corresponding hydroxylation patterns, such as kaempferol, genistein, and apigenin. However, this was not always the case; luteolin and quercetin had very different log *P* values despite their similar hydroxylation patterns.

The capacity of a flavonoid to diffuse through a plasma membrane cannot be considered similar for all flavonoids given their variation in structure and physicochemical properties (for example, equol is approximately 14 times more hydrophilic than quercetin). After removal of the sugar moiety from the flavonoid, the three-dimensional shape, isomeric structure, and number of ring substituents become the main determinants of passive diffusion. The presence of a conjugate moiety of any form aids hydrophilicity and solubility. All the monoglucosides produced log *P* values between 0.32 and 0.98; in other words, the contribution to log *P* of a flavonoid aglycon becomes insignificant once it is bound to glucose. For example, quercetin-3-glucoside and genistein-7-glucoside gave similar log *P* values despite a large difference in the log *P* of the aglycons. In the case of the sulfated flavonoids, quercetin-3-sulfate had a much lower log *P* than quercetin-7-sulfate, showing that the position of conjugation is also an important determinant of log *P*. This, presumably, is due to the intramolecular interaction of the sugar moiety with the rings and functional groups of the parent aglycon. The observation is supported by the data of Murota et al. (*11*) for quercetin glucosides. Data for quercetin 3- and 4′-glucosides and 3,4′-diglucoside (log *P* values of 1.82, 0.7, 1.32, and −1.3, respectively) suggest that although the position of

glucose conjugation is a major determinant of log *P* for these subclasses, the number of bound sugars is more influential. Each sugar molecule increases the total size, molecular weight, and number of functional groups, making the contribution of the more hydrophobic aglycon less significant. Very low log *P* values for all glycosides are evidence for their inability to be absorbed whole from the gut via passive diffusion. Recent evidence suggests that ingested glucosides are not found inside intestinal epithelial cells (*20*) or human blood plasma (*21*−*23*) and that hydrolysis is a prerequisite for diffusion. Ingested glycosides undergo enzymatic hydrolysis by lactase and β-glucosidase in the gastrointestinal tract, cleaving the glycosidic bond and leaving the aglycon to diffuse passively into the mucosal cells (*24*).

Daidzein, genistein, and equol all had relatively high log *P* values. These isoflavones/isoflavone metabolites are known for their hydrophobicity and poor water solubility. The isomeric position of the isoflavone B-ring might afford some substituent groups better protection than that of the other subclasses. Another reason could be a simple lack of substituent groups. Equol and daidzein—but not genistein—have the fewest hydroxyl groups of the flavonoids investigated here. However, genistein (log $P = 3.04$) has a markedly higher log *P* than daidzein (2.51). One explanation could be that a possible interaction between the adjacent 5-hydroxyl and the 4-ketone minimizes the hydrophilic contribution of both groups relative to the contribution of the single ketone group in daidzein. Their high lipophilicities suggest that isoflavones should have the highest affinities for lipid membranes, which could contribute to a greater degree of absorption than that of other subclasses. A relatively large amount of unchanged isoflavone is typically recovered from urine after consumption of soy foods (up to 19.8%; *25*, *26*). Methoxylation at the 6-position (and a possible interaction with the 7-hydroxyl) had the effect of lowering log *P* by an unexpected amount, since glycitein had a much lower log *P* (1.97) than the other isoflavone aglycons. Glycitein is found in soy-based products along with daidzein and genistein, but has scarcely been studied with respect to absorption, bioactivity, or estrogenicity. Daidzein and genistein are studied more widely due to their known estrogenic activity. A high lipophilicity is correlated with estrogenic activity, and equol, genistein, and daidzein have shown estrogenic activity as a consequence of their structural similarities to endogenous estrogen (*27*).

Although modeled data are more numerous and available for a wider range of flavonoids, a small number of experimental studies have previously attempted to calculate log *P* values for groups of flavonoids, each using slightly different methodologies. Cooper et al. (*19*) reported a limited number of experimental values but without reference to methodology. Murota et al. (*11*) used a shake flask technique, similar to that in the present study. Brown et al. (*28*) determined log *P* experimentally by dissolving the flavonoids in the aqueous phase, adding aliquots of octanol, and using the change in absorbance of the aqueous phase to calculate log *P*. This technique produced a notable log *P* of 0.079 for quercetin against our value of 1.82. A comparison of data obtained in the present study with modeled results is illustrated in **Figure 2**. There appears to be good agreement between the data and published modeled data (with the latter having a bias toward lower values overall). The lack of reported experimental data in the literature (which means that a meaningful correlation cannot be attempted) shows the difficulty inherent in experimental determination of log *P*. Variation in reported values shows the importance to log *P* determination of analyzing both phases.

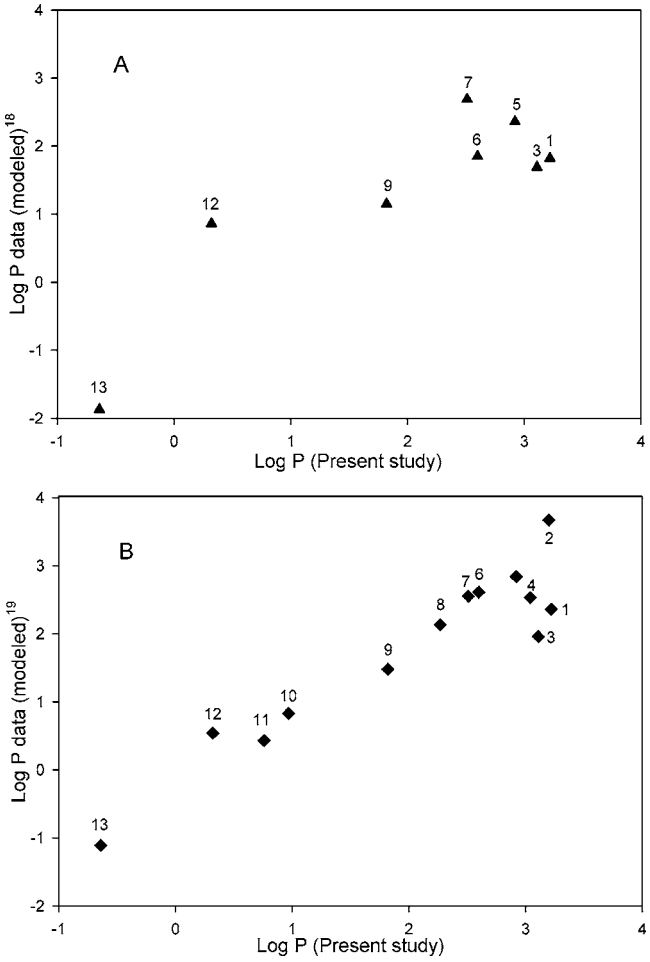

Figure 2. Comparison of experimental log *P* data determined in the present work with modeled data calculated by (**A**) Yang et al. (*18*) and (**B**) Cooper et al. (*19*). The points represent the flavonoids as follows: 1, luteolin; 2, equol; 3, kaempferol; 4, genistein; 5, apigenin; 6, naringenin; 7, daidzein; 8, eriodictyol; 9, quercetin; 10, genistein-7-glucoside; 11, quercetin-3-glucoside; 12, daidzein-7-glucoside; 13, quercetin-3-rhamnoglucoside. Flavonoids tested here but missing from the correlation were not modeled in the two studies.

log *P* determination was most difficult for the aglycons of the flavonol subclass. The log *P* value of quercetin, 1.82, was the lowest value of any aglycon in the study, although it is only sparingly soluble in water. Kaempferol, with one less hydroxyl group, was even less soluble in water than quercetin and, with mild heating, appeared to dissolve more easily in octanol. The absence of the catechol group is probably why kaempferol has a significantly higher log *P* (3.11), favoring the octanol phase. Another member of the flavonol subclass, myricetin, was observed to decompose when dissolved in water, resulting in a series of earlier-eluting peaks on the chromatogram. Myricetin has three adjacent hydroxyl groups on the B-ring, and ring fission may occur as a consequence of reacting with water, producing lower molecular weight derivatives. It is apparent that the complex spatial arrangement of hydroxyl groups on the flavonol skeleton imparts unpredictable behavior with respect to water. Coefficients of variation for quercetin and kaempferol were high (5−10-fold higher than those of most other compounds); this was largely due to differences in the flavonol concentration in the aqueous phase among replicates. These difficulties raise the question of whether it is possible to obtain precise values experimentally for some compounds if they are not freely soluble, and stable, in both

octanol and water. The method of log *P* determination is limited in that the analyte must be completely dissolved, and stable, in one phase before the other is added. Quercetin shows unusual behavior among flavonoids. It is slightly soluble in both solvents, once dissolved is reluctant to stay in solution, and will precipitate readily, often to a state that cannot be redissolved. The low log *P* of quercetin aglycon could have important consequences with respect to in vivo fate. It has been established that the lack of solubility hinders intestinal absorption, and therefore inhibits plasma accumulation in rats (*29*). Therefore, the behavior of quercetin in vivo might be significantly different from that of structurally similar aglycons as well as glycosides, occupying a unique position in flavonoid physicochemical reactivity. It is speculated that the properties imparted by the 3-hydroxyl of the flavonols, although they might give rise to many types of bioactivity, may contribute to the low extent of absorption of quercetin from the gastrointestinal tract.

In the present study, the amount of flavonoid in both the octanol and buffer fractions was measured, and the average recovery was calculated to check that large amounts of compound were not unaccounted for. The difficulties observed in recovering a high percentage of compound confirmed that, for an accurate determination of log *P*, it is absolutely necessary to measure the content of both fractions. Sometimes the order of log *P* values among flavonoids disagrees with expectation (luteolin, equol, and daidzein, for example), but the relative mobilities of each of the compounds on the HPLC column also show unexpected differences. A higher log *P* does not always imply an increased retention time; quercetin aglycon is retained on the column longer than luteolin, despite having a log *P* value 1.5 units lower.

The importance of established physicochemical data for dietary flavonoids should not be underestimated. To understand the ways in which different flavonoids are absorbed, it is necessary to first examine the underlying molecular properties that determine interactions with cells and tissues. It cannot be assumed that all flavonoids, or even all of a particular flavonoid subclass, behave in the same way after ingestion, and after absorption. log *P* data contribute to our understanding, both alone and together with data describing other molecular characteristics which are related to or pertain to in vivo bioactivity.

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