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ARTICLES

Gas-Phase Ligation of Fe^+ and Cu^+ Ions with Some Flavonoids

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It was assumed that gas-phase ligation of metal monocations by flavonoids might provide some insight on the intrinsic antioxidant activity of the latter. Thus, the ligation of Fe^+ and Cu^+ ions by apigenin (**1**), luteolin (**2**), kaempferol (**3**), quercetin (**4**), myricetin (**5**), and naringenin (**6**) was investigated in the gas phase in a Fourier transform mass spectrometer (FTMS). Both of the metal ions, which were produced by laser desorption ionization (LDI), bind consecutively to two neutral flavonoid molecules either with or without the simultaneous loss of some part (H, CO, H_2O) of the latter. The flavonoids are present in the instrument at steady concentrations. The formation of flavonoid positive ions by charge exchange is also a common observation but is accompanied, in some cases, by a loss of H, CO, or H_2O fragments. The reaction paths and observed fragmentations are presented. The results are supported by DFT B3LYP calculations that indicate a preference for metal ion attack at C-ring and not at the B-ring site considered to be mainly responsible for flavonoid antioxidant activity.

KEYWORDS: Flavonoids; antioxidants and antiradical activity; DFT calculation; FTICR MS; ligation and fragmentation products with Fe^+ and Cu^+

INTRODUCTION

The flavonoids (Fl) of interest belong to three antioxidant subgroups: apigenin (**1**) and luteolin (**2**) are flavones; kaempferol (**3**), quercetin (**4**), and myricetin (**5**) are flavonols; and naringenin (**6**) is a flavanone (**Scheme 1**).

We have chosen these compounds because our recent investigation of their reactions with radicals showed great variations of activity (*1*). However, it is known that flavonoids, when tested by methods based either on the quenching of reactive oxygen species (*2*−*6*), on reactions with synthetic radicals (*7*−*10*), or on enzymatic and nonenzymatic measurement of lipid peroxidation inhibition (*11*, *12*), may exhibit quite different activities. The activity in solution, liquid, and solid phases is based on an electron transfer from Fl to the oxidant (or radical species), in which transfer occurs either simultaneously with a proton transfer (i.e., an H-atom transfer mechanism, HAT (*13*)) or the proton transfer follows later via a solvent molecule (i.e., single electron-transfer mechanism, SET (*13*)). It seemed of interest to investigate the immediate consequences of a complete transfer of a flavonoid molecule to a strong oxidant, such as a singly charged transition metal cation in the gas phase. Such results might provide some insights on the intrinsic activities of flavonoids, ones that are not dependent on solvent or other molecule participation. On the other hand, one of the flavonoid antioxidant actions is an interaction with metal ions during their reactions with free radicals. Iron and copper ions play an important role in the Fenton reaction, which yields hydroxyl radicals (*14*−*18*). In Fenton reactions, the lipid hydroperoxides decompose into alkoxy radicals, which either support the lipid peroxidation chain reaction or react with biological cell material. The use of flavonoids instead of other potentially harmful synthetic compounds was suggested for binding the metals in such situations.

EXPERIMENTAL PROCEDURES

Compounds. Apigenin, kaempferol, and myricetin were obtained from Fluka (Buchs, Switzerland), and luteolin, quercetin, and naringenin were from Roth (Karlsruhe, Germany). Metal ions were produced from a stainless steel target or from foils of high 99.8% purity (Achesson Industries Ltd.).

Experiments. All experiments were performed on a Finnigan FT/MS 2001-DD Fourier-transform mass spectrometer (Madison, WI) equipped with a 3 T superconducting magnet and Nd:YAG Quanta Ray DCR-11 laser (Spectra-Physics Inc., Mountain View, CA).

The gas-phase reactions of Fe^+ or Cu^+ ions with a flavonoid were carried out in the FTMS instrument. The metal ions were produced by a single laser pulse, LDI, and were confined in circular motion in the

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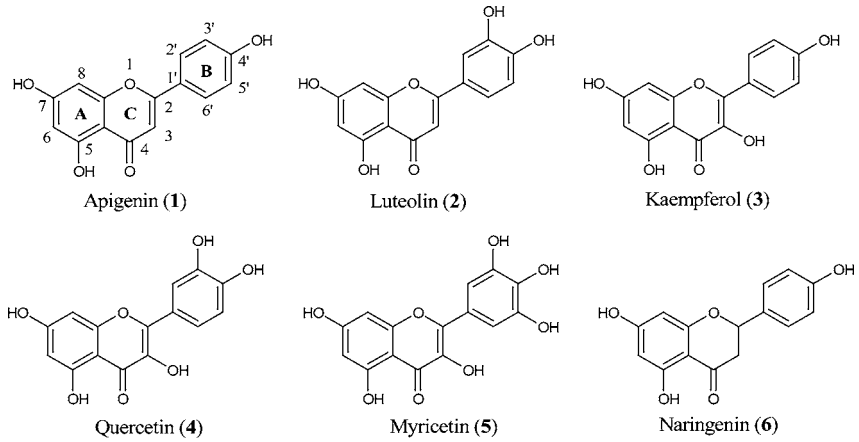
Scheme 1. Structures of Flavonoids



analyzer cell. A steady concentration of the neutral flavonoid was maintained in the same cell by thermal evaporation, the whole instrument being maintained at 250 °C. After a reaction time of about 100 ms, which allowed the disappearance of electronically excited ions, all ions other than iron-56 or copper-63 isotope ions were removed from the cell and the reaction was again allowed to proceed.

All positively charged ions were then recorded via Fourier transformation of their characteristic frequencies of rotation in the cell. Several such snapshots at various delay times after the new reaction starts allow one to follow the type and relative rates of products formation.

Calculations. All calculations were performed using Becke's three-parameter hybrid exchange functional (B3) (*19*) together with the correlation functional of Lee, Yang, and Parr (LYP) (*20*) and the 6-311++ G(d,p) basis set with the Gaussian 03 program (*21*). In the calculations, previously optimized structures of flavonoids were "attacked", that is, approached by a Fe^+ or Cu^+ ion from various directions, and the resulting optimized stabilized structures yielded the most probable one as that with the obtained lowest energy.

RESULTS AND DISCUSSION

Metal ions, M^+, together with the neutral molecules of Fl, all in the gas phase within the spectrometer, constitute a dynamic

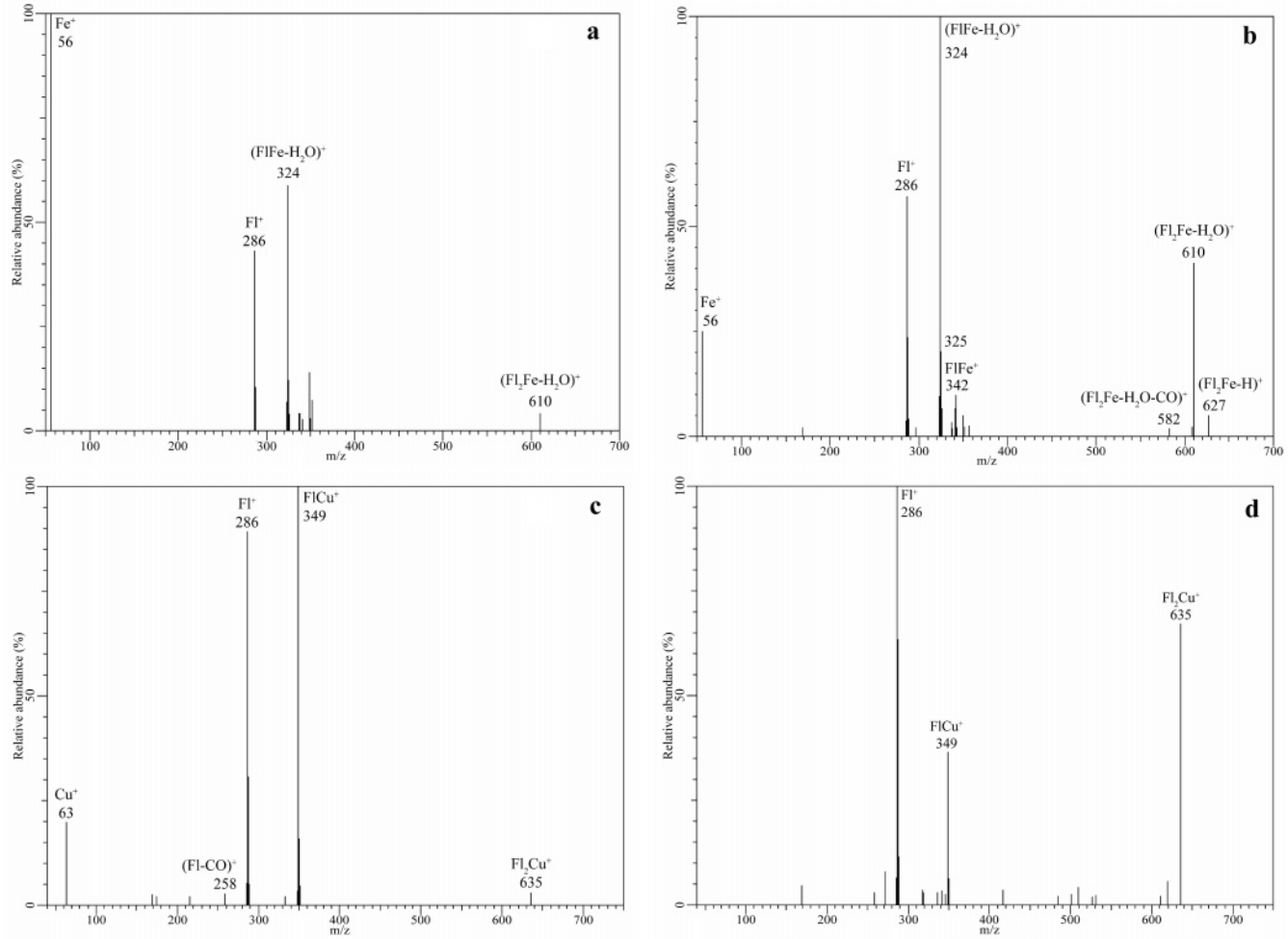


Figure 1. Mass spectra of keampferol with Fe^+ at delay times of (**a**) 1 ms and (**b**) 100 ms, and those of kaempferol with Cu^+ at delay times of (**c**) 200 ms and (**d**) 1 s.

Table 1. Observed Reaction Products of Flavonoids **1**–**6** with Fe^+ Ions

flavonoid	$Fl^{+-}X$ (X = nil, H, H_2O, CO)	$FlFe^{+-}X$ (X = nil, H, H_2O, CO)	$Fl_2Fe^{+-}X$ (X = nil, H, H_2, H_2O, CO)
apigenin (**1**)	(nil), (H), (CO)	(nil), (H), (CO)	(nil), (H), (H_2), (H_2O)
luteolin (**2**)	(nil), (H), (CO)	(nil), (H), (CO)	(H), (H_2), (H_2O)
kaempferol (**3**)	(nil)	(nil), (H_2O)	(H), (H_2O), (H_2O and CO)
quercetin (**4**)	(nil)	(nil), (H_2O)	(H), (H_2O)
myricetin (**5**)	(nil), (H), (H_2O), (CO)	(nil), (H_2O)	(H), (H_2O)
naringenin (**6**)	(nil), (H), (H_2O), (H_2O and CO)	(nil), (H_2O and CO)	(nil), (H_2O), (H_2O and CO)

system. This system may form complexes by the reaction:

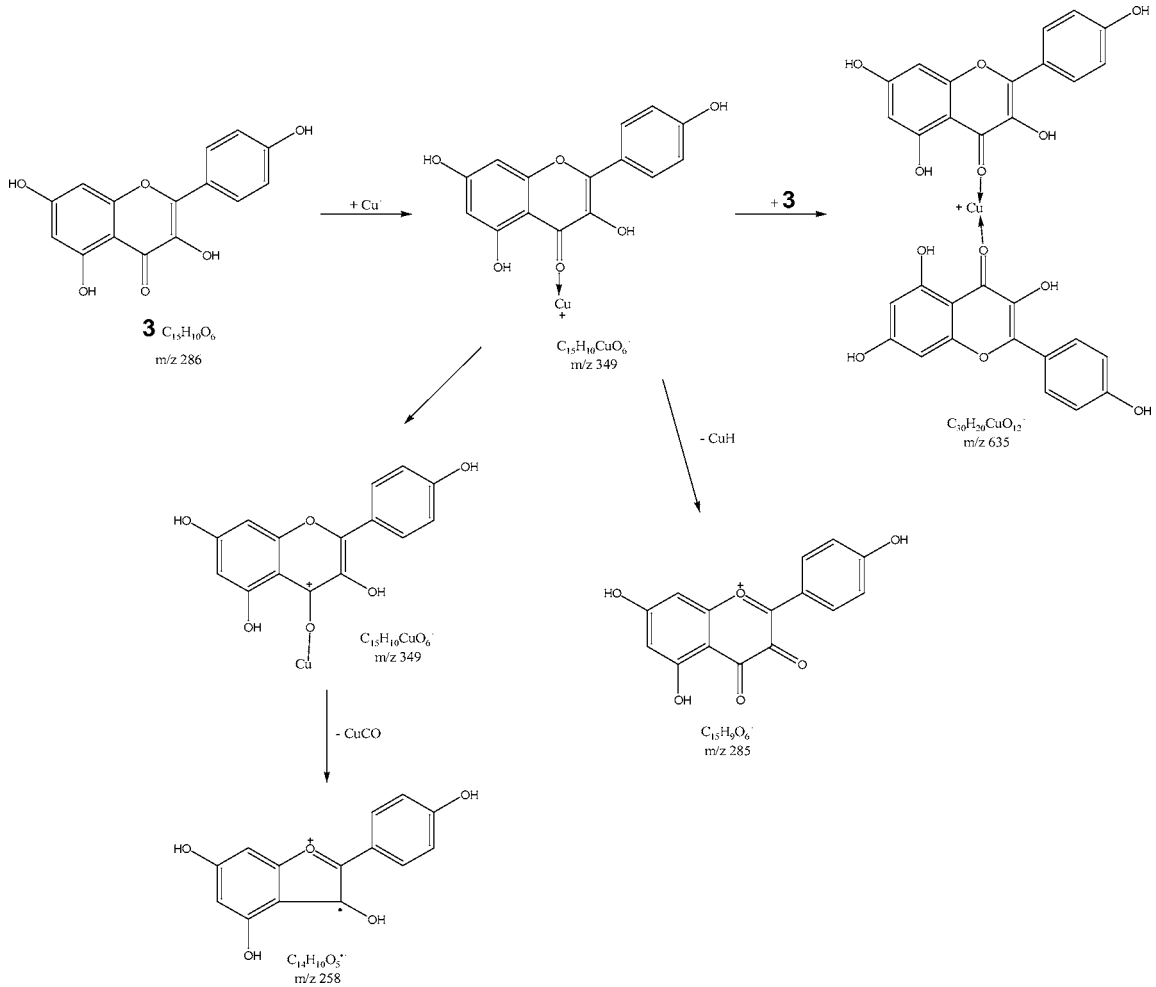
$$M^+ \xrightarrow[-X']{+Fl} (MFl-X')^+ \xrightarrow[-X'']{+Fl} (MFl_2 - X' - X'')^+ \text{ etc.}$$

$$X',X'' = \text{nil, H, CO, } H_2O$$

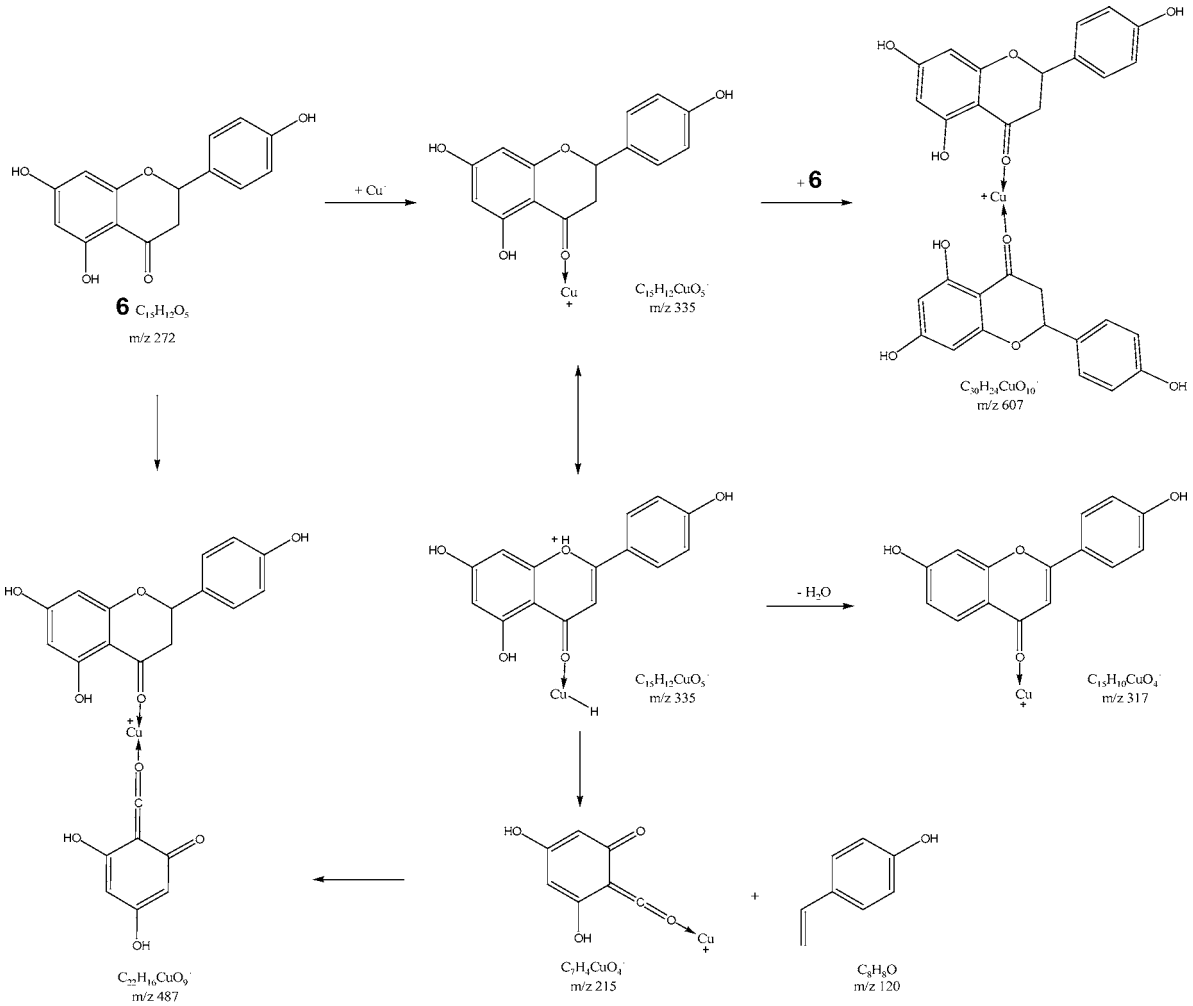
Other reactions may also occur (e.g., trace amounts of water participate in proton transfer and in the protonation of neutral Fl molecules). C−C bond cleavage during organometallic complex formation is much less common in solution than in the gas phase where the activated complex carries a considerable surplus of internal energy; in specific, this surplus, when combined with the energy released from the newly formed metal−carbon bond, is sufficient to break the C−C bond of the ligand (*22*). This energy surplus is also the reason for observation of a considerable amount of charge transfer, although the ratio of ionization potentials between the metals (Fe = 7.87 eV, Cu = 7.726 eV) and the ligands (all Fl have IP's of ∼7−8 eV) would suggest it. The complexes of M^+ and Fl form in two ways: by simple addition and by substitution accompanied by expulsion of water or some other small part of Fl. In the latter case, the reaction involves activation of a C−X (X = H, O, or C) bond in the ligand. For M^+ attachment to the ligand, the following steps may be considered: oxidative addition, functional group or hydrogen transfer from the β-position of the ligand molecule, and reductive elimination. Oxidative addition yields an intermediate in which two new bonds with the metal are formed; therefore, at least two nonbonding metal electrons are required. Thus, the difference of electronic structure between Fe^+ and Cu^+ is the primary reason for their different reactivities with the Fl molecules. The mass spectra of kaempferol with Fe^+ at delay times of 1 and 100 ms are shown in **Figure 1a** and **b**, and those of kaempferol with Cu^+ at delay times of 200 ms and 1 s in **Figure 1c** and **d**, respectively.

Reactions with Fe^+ Ions. The initial interaction of Fe^+ and Fl leads to formation of an activated complex with sufficient internal energy for reaction. This energy surplus enables a charge exchange reaction. In **Table 1**, the observed reaction products are shown. In all reactions, a positively charged Fl^+ is formed; indeed, all flavonoids of the same group show similar reactivities and types of reaction products. The presence or absence of a double bond and a hydroxyl group in the 3-position (C-ring) of

Scheme 2. Formation and Fragmentation Processes of Cu^+ with Kaempferol (**3**)



Scheme 3. Formation and Fragmentation Processes of Cu^+ with Naringenin (**6**)



Fl determines the reaction path and nature of the products. In all experiments, Fe^+ reacts either by simple addition or by substitution reaction through C−C bond activation; however, if that carbon possesses a hydroxyl group, then C−H and C−O bond activation is observed.

Molecules **1** and **2**, which have a 2,3-double bond but no adjacent hydroxyl group, form Fl substitution complexes by release of hydrogen (i.e., by ligand oxidation). This behavior accords with complex formation in solution, in which these flavonoids are oxidized in a reaction with metal ions (*23*, *24*). The loss of CO can result in considerable structure change. Similarly, in a fraction of the observed charge exchange products, a neutral iron carbonyl fragment can be expelled from the complex. The characteristics of the second group (i.e., of compounds **4**, **5**, and **6**) are a loss of water instead of CO upon chelation and no loss at all from the ligand during charge exchange. Compound **6** is the most susceptible to fragmentation in all reactions: both CO and H_2O are lost from the complex and from the Fl^+ ions. Prolonged reaction times yield reaction products that contain an additional Fl molecule. Only compounds **1**, **2**, and **6** show the simple double addition products Fl_2Fe^+ along with those formed by loss of H or H_2O; in **3** and **4**, the sole double ligation products are mainly $(Fl_2Fe-H_2O)^+$ and little $(Fl_2Fe-H)^+$.

Reactions with Cu^+ Ions. Comparison of the products formed in the gas-phase reactions of neutral flavonoid molecules with Cu^+ and with Fe^+ constitutes a nice example of the way in which the electron configuration of the metal cation affects the reaction path. The copper monocation tends to preserve its +1 oxidation state because that configuration assures stable 10 paired electrons in the 3d subshell. Consequently, Cu^+ will avoid oxidative addition upon insertion into a ligand bond because this would disrupt subshell completion. Oxidative addition of copper ions in solution takes place in a very specific way by formation of a dimer within the reaction complex (*25*). Such dimerization enables a one-electron oxidation pathway for the metal ion by avoiding the unstable Cu(III) oxidation state. However, in the gas phase, dimer formation is not probable, and, therefore, Cu^+ reacts exclusively by binding hydride and negatively charged alkyl, hydroxyl, or other groups from neighboring C-atoms and, in this manner, avoids activation of any bond.

The observed reaction products are given in **Table 2**. Compounds **1** and **2** of the first group, in their reactions with Cu^+, yield only addition products in both reaction steps, and charge transfer proceeds without any loss from the ligand. The second group (i.e., compounds **3**, **4**, and **5**) shows somewhat different reactivity. The complexes formed in both steps are solely of an addition type, indicating a lack of reaction by ligand bond activation. However, there are some ion signals present that derive from transfer of a hydride unit from neighboring hydroxyl groups and expulsion of the copper in the form of

Table 2. Observed Reaction Products of Flavonoids **1**–**6** with Cu^+ Ions

flavonoid	$Fl^{+-}X$ (X = nil, H, H_2O, CO, $^{1,3}B$)	$FlCu^{+-}X$ (X = nil, H_2O, CO, $^{1,3}B$)	$Fl_2Cu^{+-}X$ (X = nil, $^{1,3}B$)
apigenin (**1**)	(nil)	(nil)	(nil)
luteolin (**2**)	(nil)	(nil)	(nil)
kaempferol (**3**)	(nil), (H), (CO)	(nil)	(nil)
quercetin (**4**)	(nil), (H)	(nil), (H_2O), (H_2O and CO)	(nil)
myricetin (**5**)	(nil),(H), (H_2O), (H_2O and CO)	(nil), (H_2O), (H_2O and CO)	(nil)
naringenin (**6**)	(nil), (H), (H_2O), ($^{1,3}B$)	(nil), ($^{1,3}B$)	(nil), ($^{1,3}B$)

Table 3. Calculated Difference in Energy (kcal/mol) for the Attachment of Metal Ion to the Next Preferred Reaction Site in Flavonoids **1**, **4**, and **6**

flavonoid	Cu^+/kcal/mol
apigenin (**1**)	29.5
quercetin (**4**)	14.2
naringenin (**6**)	19.8

neutral copper hydride. In another reaction, copper attracts the neighboring hydroxyl group, which results in the expulsion of a water molecule. By transfer of the hydroxyl group to the metal center, the positive charge remains at the C-ring carbon atom, which together with carbonyl group enolization by Cu^+ yields a sigmatropic rearrangement and a release of CO from the flavonoid in the form of copper carbonyl. These processes are shown in **Scheme 2** for kaempferol.

In the reaction of **6** with Cu^+, addition complexes are formed again in both reaction steps. In the first, either a release of one water molecule or a *retro*-Diels−Alder (RDA) reaction occurs; the reaction intermediate with a double bond in the C-ring probably forms by transfer of hydride from a neighboring carbon to the copper ion, thus inducing a positive charge on the C-ring and causing proton transfer to its oxygen (**Scheme 3**). The *retro*-Diels−Alder (RDA) reaction pathway yields in addition to its characteristic products also a ligation product with the ligand (*m*/*z* 487).

The reactivity and biological activity of Fl have been investigated by several empirical and qualitative methods, and, as a result, it is clear that it is impossible to determine quantitatively or predict them theoretically in any reliable way. Because these properties develop in environments under conditions that are difficult to determine and control, it is not surprising that the activities determined by various methods correlate poorly and are not reliable. For example, antioxidative activity is governed by the radical-scavenging rate constants, the stability of the respective phenoxyl radicals formed from the antioxidant molecules, and their univalent redox potentials (*26*, *27*). If needed, all of these parameters have to be determined in different environments by respective methods and yield a distorted picture of the antioxidative property. We had hoped that the simple, gas-phase addition of metal ions to flavonoids would take place at the same reactive site that is also responsible for antioxidant activity, that is, at a hydroxyl group of the B-ring. If so, that would have provided results of Fl behavior on such attack without the presence of solvent effects. We did DFT and molecular dynamics calculations for representative flavonoids **1**, **4**, and **6**, which indicate a preference for the metal ion to sit close to the carbonyl oxygen at position 4 pointing toward ring B even when there is no substituent in position 3. The difference in energy to the next favorable attachment site of the metal ion which is either the oxygen atom in 4′-position or between the two oxygen atoms in 3′,4′-positions is given in **Table 3**; in both of the two latter structures the corresponding hydrogen atoms point away from the metal. The energy difference for these two best sites for attack favors the C-ring location. This result helps one to understand the mass spectra and reaction products in some detail (**Figure 1**), but because of the relatively small energy difference leaves open the possibility that to some extent attachment and fragmentation may proceed also at hydroxyl group(s) of ring B. However, the result indicates that the intrinsic reactivity of flavonoids at least with here investigated metal ions differs from that they show while exhibiting their biological activity. However, such investigations do provide a means of understanding the intrinsic properties of flavonoid molecules, and we will continue to pursue them.

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