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Competition between Ascorbate and Glutathione for the Oxidized Form of Methylated Quercetin Metabolites and Analogues: Tamarixetin, 4′O-Methylquercetin, Has the Lowest Thiol Reactivity

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Supporting Information

ABSTRACT: Quercetin (**Q**) is a bioactive compound with excellent antioxidant activity. However, the thiol reactivity of its oxidation product (**oxQ**) forms a disadvantage. The aim of the present study was to decrease this thiol toxicity. We found that methylated **Q** metabolites displayed lower thiol reactivity than **Q**. The most effective was tamarixetin, 4′O-methylquercetin (**4′MQ**), that has a corresponding oxidation product (**ox4′MQ**) with thiol reactivity 350 times lower than **oxQ**. The endogenous metabolism of **Q** to **4′MQ** might be a physiological way to safely benefit from the antioxidant potential of **Q** in vivo. Our results were explained with Pearson's HSAB concept and corroborated by quantum molecular calculations that revealed a strong correlation between the relative thiol reactivity and the lowest unoccupied molecular orbital (LUMO). The polarity of the molecule and the $\pi-\pi$ interaction between the AC- and the B-ring appeared to determine the LUMO and the thiol reactivity of the oxidation product.

KEYWORDS: *antioxidant, flavonoid, quercetin, methylation, metabolite, glutathione, ascorbate*

INTRODUCTION

Flavonoids are important nutrients ubiquitously found in our diet, and they are also used as nutraceuticals.[1] Some of them, with quercetin (**Q**) as the best studied example, display an excellent antioxidant activity. However, the reactive oxidation products that are formed out of flavonoids, i.e. electrophilic quinones, form a disadvantage. These quinones are harmful because they readily react with vital thiol groups.[2] To protect against quinone toxicity, cells are endorsed with an intricate antioxidant network. The most prominent constituents of this network that can protect against quinone toxicity are ascorbate and glutathione (GSH).[3] It should be noted that both antioxidants neutralize quinones differently. Ascorbate converts quinones back to the parent flavonoid, whereas GSH reacts with quinones to form a GSH-flavonoid adduct. The reaction of oxidized **Q** (**oxQ**) with GSH is much faster than with ascorbate.[4] When GSH is depleted, thiol-containing proteins become the new targets for the electrophilic **oxQ**. The reaction with thiol-containing proteins will cause toxicity. In addition, GSH might only offer partial protection since GSH-Q adducts rapidly react with protein-thiols at physiological conditions, hence, spreading the toxicity over the cells.[5]

To circumvent thiol toxicity, we aim to identify quercetin derivatives that would preferentially react with ascorbate rather than with thiols. Besides preventing toxicity, an additional advantage is that, in the reaction with ascorbate, the parent flavonoid is recycled which enhances the antioxidant activity of the flavonoid. We tested a series of methylated **Q** derivatives that included two of the major metabolites of **Q** formed in vivo, i.e. tamarixetin (**4′MQ**) and isorhamnetin (**3′MQ**). These metabolites are held responsible for at least part of the health benefits of **Q**.[6−8] We determined the competition between ascorbate and thiols (CAT) for the oxidized flavonoids. GSH was used as thiol and the CAT was calculated as the reaction rate of oxidized flavonoid with ascorbate (V_{Asc}) divided by the reaction rate of the oxidized flavonoid with the thiol GSH (V_{GSH}). Our final goal is to identify the structural elements that will direct the reactivity of the oxidized product toward ascorbate instead of thiols, in other words, to increase the CAT. With this knowledge, strategies might be developed that selectively direct the reactivity of free radicals more safely into the endogenous cellular antioxidant network.

MATERIALS AND METHODS

Chemicals. Quercetin·$2H_2O$ was purchased from Acros Organics and rutin·$3H_2O$ from Sigma-Aldrich (Steinheim, Germany). The methylated derivatives of quercetin (Table 1) were synthesized and can be currently purchased (www.maxamed-chem.com). Glutathione (GSH), hydrogen peroxide (H_2O_2), horseradish peroxidase (HRP), and L-ascorbic acid (vitamin C) were purchased from Sigma (St. Louis, MO). Trifluoroacetic acid (TFA) was acquired from Sigma-Aldrich (Steinheim, Germany). Acetonitrile, HPLC grade, was obtained from Biosolve (Valkenswaard, The Netherlands).

Oxidation of the Flavonoids. All solutions were freshly prepared and used within three hours. The flavonoids were dissolved in DMSO. GSH, vitamin C, and H_2O_2 were dissolved in 145 mM phosphate buffer (pH 7.4). In the incubations, the reactants, i.e. flavonoid, GSH, vitamin C, and H_2O_2 were used at equimolar concentrations (50 μM), unless otherwise noted. The oxidation reaction was performed at 37 °C in 145 mM phosphate buffer (pH 7.4) and started with the addition of HRP. HRP was used in a concentration that oxidized the

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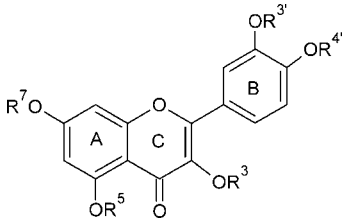
Table 1. Competition between Ascorbate and Thiols (CAT) for the Oxidized Flavonoid



compd	R^3	R^5	R^7	$R^{3'}$	$R^{4'}$	CAT
Q	H	H	H	H	H	0.04 ± 0.03
5MQ	H	CH_3	H	H	H	0.11 ± 0.05
7MQ	H	H	CH_3	H	H	0.24 ± 0.03
3′MQ	H	H	H	CH_3	H	0.75 ± 0.13
3MQ	CH_3	H	H	H	H	1.77 ± 0.20
3,7MQ	CH_3	H	CH_3	H	H	1.93 ± 0.19
3RuQ	Ru[a]	H	H	H	H	2.90 ± 0.51
4′MQ	H	H	H	H	CH_3	14.5 ± 3.8

[a]Ru is a rutinosyl group that is a disaccharide consisting of glucose and rhamnose.

flavonoid under investigation at a rate of approximately 50% flavonoid in 5 min at 37 °C. The reactions were monitored spectrophotometrically and by HPLC.

Spectrophotometric Analysis. Spectrophotometric analysis was performed with a Varian Carry 50 spectrophotometer (Varian, Mulgrave, VIC, Australia). All absorption spectra were recorded from 200 to 500 nm with a scan speed of 600 nm/min, using quartz cuvettes. The UV/vis scans were started 15 s after the addition of HRP, and the reaction was monitored for 10 min at 37 °C.

HPLC Analysis. High-performance liquid chromatography (HPLC) was performed using a HP 1100 series HPLC system (Agilent Technologies, Palo Alto, CA). Analytical separations were achieved using Alltima HP C18 column (3 μm, 15 cm × 3.0 mm). The mobile phase consisted of water containing 0.1% (v/v) TFA with a linear gradient from 5% acetonitrile to 20% acetonitrile in 5 min, followed by an increase to 60% acetonitrile at 10 min. A flow rate of 1 mL/min was used. Detection was carried out with a diode array detector (DAD). The HPLC measurements were performed 5 min after the reaction was started.

Competition between Ascorbate and Thiols for the Oxidized Flavonoid. The competition between ascorbate and thiols for the oxidized flavonoid, the CAT, is determined as the rate of reaction of oxidized flavonoid with ascorbate (V_{Asc}) divided by the rate of the reaction of the oxidized flavonoid with the thiol GSH (V_{GSH}), when both ascorbate and GSH are present at an equimolar concentration:

$$CAT = V_{Asc}/V_{GSH}$$

The concentrations of GSH and ascorbate vary within the body. Typically, GSH concentrations in the cells are in the millimolar range whereas in blood plasma GSH is practically absent. Ascorbate concentrations range from 40 to 60 μM in blood plasma up to the millimolar range in cells. The concentrations used for the competition experiment were 50 μM GSH and 50 μM ascorbate. GSH is known to react with oxidized flavonoids to give GSH-flavonoid adducts, and in this reaction, GSH-flavonoid adduct formation equals flavonoid consumption. Ascorbate reduces oxidized flavonoids to the parent flavonoid, but it can also partially inhibit HRP activity as reported previously.[4] Both activities of ascorbate will prevent net consumption of the flavonoid. So, in the competition between GSH (50 μM) and ascorbate (50 μM) for the oxidized flavonoids, ascorbate has two non-negligible activities: (i) reduction of the oxidized flavonoid to the parent flavonoid, (ii) inhibition of the rate of flavonoid oxidation by inhibiting HRP activity. When GSH is in excess (10 mM) compared to ascorbate (50 μM), the rate of the oxidized flavonoid with ascorbate is negligible to that with GSH. Hence, all the oxidized flavonoids will be trapped by the excess of GSH, and the 50 μM ascorbate can only protect against flavonoid consumption by inhibiting the HRP enzyme. The rate of oxidation of the flavonoid under this condition, $V_{oxidation}$, was assessed by measuring the consumption of the flavonoid using HPLC. Similarly, flavonoid consumption by HRP in the presence of 50 μM GSH and 50 μM ascorbate was used to determine V_{GSH}. V_{Asc}, the rate of the reaction of the oxidized flavonoid with ascorbate in the competition experiment with 50 μM GSH and 50 μM ascorbate, was calculated by subtracting V_{GSH} from $V_{oxidation}$:

$$V_{Asc} = V_{oxidation} - V_{GSH}$$

Using the procedure described in this section, we were able to correct for the inhibition of HRP by 50 μM ascorbate. Although V_{asc} and V_{GSH} are presented as absolute rates, they represent relative values where both rates are relative to each other. The rate limiting step is the oxidation of the flavonoid by HRP/H_2O_2 that generates the quinone. Subsequently, the quinone reacts very quickly with either GSH or ascorbate. Because V_{asc} and V_{GSH} are related to each other, we used their ratio, i.e. CAT.

Quantum Molecular Calculations. Quantum molecular calculations were performed using Spartan'10 (Wavefunction, Irvine, CA) to determine the relative abundance of the tautomers of the oxidized flavonoids. Subsequently, the lowest unoccupied molecular orbital, LUMO, energies (kJ/mol) of the most abundant tautomers were calculated as well as their equilibrium geometry. The latter was used to determine the dihedrals (φ), the angles between the plane of the AC-ring and that of the B-ring in the oxidized flavonoids. Previous studies have shown that ab initio calculations using the Hartree−Fock (HF) 3-21G basis set provides sufficiently good results for estimating chemical "hardness",[9] and therefore this was used.

Statistics. All experiments were performed, at least, in triplicate. Data are expressed as means ± SD or as a typical example.

RESULTS

GSH Reacts with Oxidized Quercetin To Form GSHQ Adducts. UV analysis shows that the reaction of **Q** with HRP/H_2O_2 leads to a decrease of the absorbance at 380 nm (λ_{max} of **Q**) that indicates the oxidation of **Q** (Figure 1A). When **Q** is oxidized in the presence of GSH, isosbestic points in the UV spectrum at 308 and 354 nm are seen (Figure 1B) that clearly differ from those without GSH, i.e. 284 and 364 nm (Figure 1A). HPLC analysis confirms that in the presence of GSH, two **GSH-Q** adducts are formed that were previously identified as the **6**- and the **8-GSQ** adducts (Figure 2).[4]

Ascorbate Regenerates Oxidized Q to Q. Addition of ascorbate to the incubation mixture containing **Q** and HRP/H_2O_2 prevents the consumption of the flavonoid, since the absorbance between 300 and 400 nm due to **Q** remains unchanged (Figure 1C). At the same time, ascorbate is consumed, evidenced by the decrease in absorbance at 270 nm (λ_{max} of ascorbate). A control experiment showed that when **Q** is omitted from the incubation mixture, there is no detectable ascorbate consumption. This indicates that ascorbate does not react with HRP/H_2O_2. Therefore it is concluded that **Q** is oxidized by HRP/H_2O_2 and is then immediately regenerated by ascorbate. HPLC analysis confirms that there is no net **Q** consumption when ascorbate is present, within the 5 min of incubation.

Competition between Ascorbate and Thiols for the Oxidized Flavonoid (CAT). The CATs of the flavonoids were quantitatively determined using HPLC analysis. As a typical example, V_{GSH} with **oxQ** was 4.6 μM/min while V_{Asc} was only 0.2 μM/min. This means that the reaction rate of **oxQ** with ascorbate is much lower than the reaction rate of **oxQ** with GSH, which is reflected by a low CAT of 0.04 (Table 1).

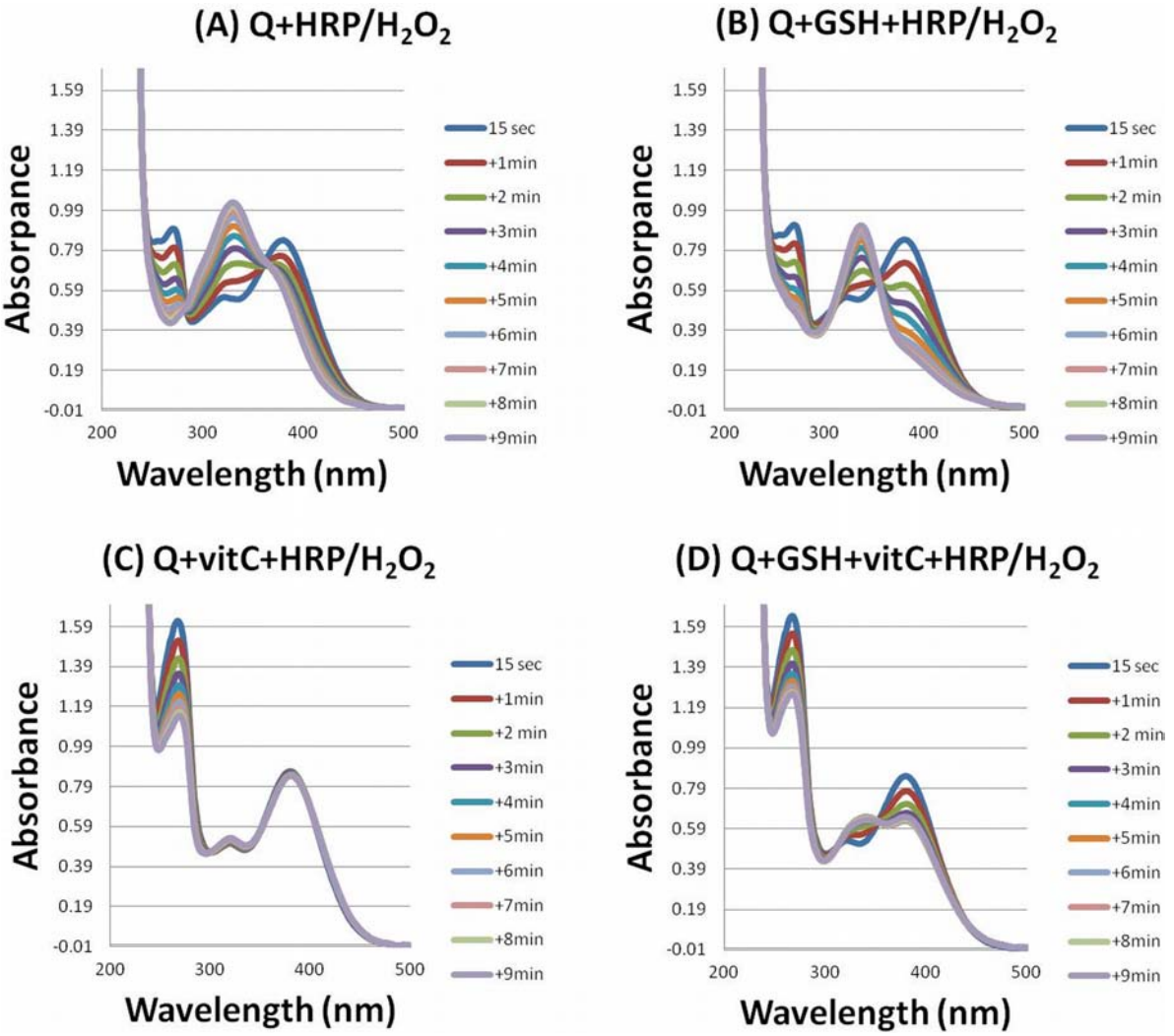


Figure 1. UV scans of the incubation mixture containing 1.6 nM HRP, 50 μM quercetin, and 50 μM H_2O_2 (A). The same experiment was carried out in the presence of 50 μM GSH (B) or 50 μM ascorbate (C), and in the presence of both 50 μM ascorbate and 50 μM GSH (D). The UV scans were started 15 s after the addition of HRP and had a speed scan of 600 nm/min. After each minute one scan was taken with a total of 10 scans per reaction.

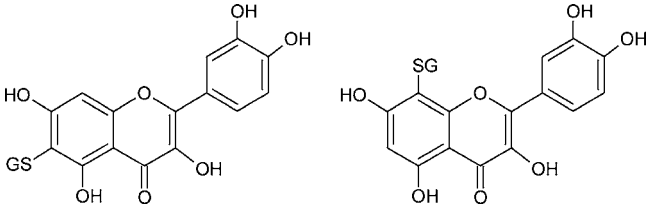


Figure 2. GSH adducts of **Q**. On the left is depicted **6-GSQ**, and on the right, **8-GSQ**.

Similarly to **Q**, the derivatives of **Q** are oxidized by HRP/H_2O_2. The oxidation products are regenerated in the presence of ascorbate and form GSH-adducts in the presence of GSH. The CATs are depicted in Table 1. It was found that methylation increased the CAT. Methylation of the 4′OH group had the most profound effect; it increased the CAT of **Q** from 0.04 to 14.5.

Quantum Molecular Calculations. The oxidized flavonoids have four different tautomers (T_1–T_4) as illustrated in Figures 3 and 4 for some of these compounds. Quantum molecular calculations, performed as described in the Materials and Methods section, revealed that for **oxQ** the T_3 tautomer is by far the most favorable one with an abundance of more than 99%. For **ox5MQ**, **ox3′MQ**, and **ox4′MQ** this also is T_3. For **ox7MQ** this is T_4, for **ox3MQ**, **ox3RuQ**, and **ox3,7MQ** this is T_1.

OxQ is planar with a dihedral between the plane of the AC-ring and that of the B-ring of 0.0°. Methylation at the 3O-position increases this dihedral (Table 2). A methyl group at the 3O-position as in **ox3MQ** and **ox3,7MQ** induces an angle of 13°. The more bulky rutinosyl group in **ox3RuQ** increases the dihedral to 29°.

The energies of the LUMOs of the most abundant tautomer of **oxQ**, its methylated derivatives, and **ox3RuQ** are depicted in Table 1. **OxQ** has the lowest LUMO energy and O-methylation lead to an increase of the LUMO energy. **Ox4′MQ** has the highest LUMO energy of the methylated derivatives. It was found that the LUMO energies highly correlated (R^2 = 0.9713) with the CATs, as illustrated in Figure 5. The relationship, which covered a wide range of CAT, was

$$\ln(\mathrm{CAT}) = 0.0407\mathrm{LUMO} + 1.5736$$

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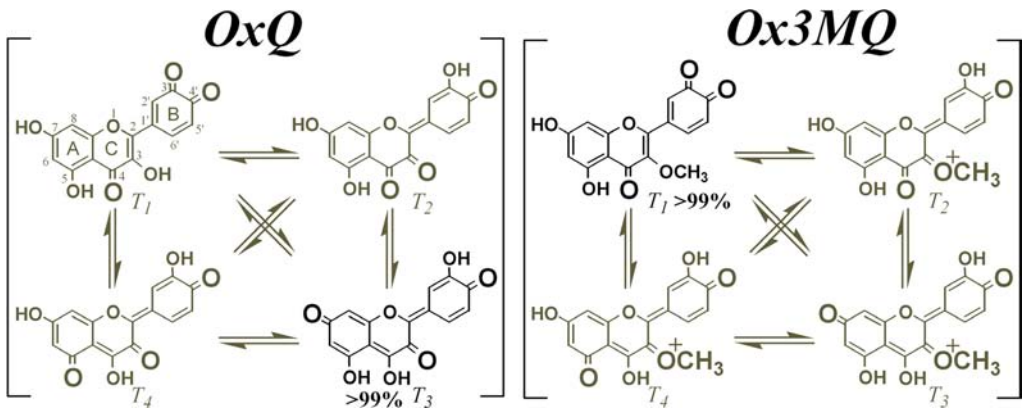

Figure 3. Tautomers of oxidized **Q** (**oxQ**) and oxidized **3MQ** (**ox3MQ**). For **oxQ**, T_3 is the most favorable tautomer because the carbonyl groups are at maximum distance from each other. For **ox3MQ**, T_1 is the only tautomer that is not charged making it the most energetically favorable configuration. The other tautomers of **ox3MQ**, T_2–T_4, are positively charged making them energetically less favorable. Quantum molecular calculations indicate that the preferred tautomer of both compounds has a relative abundance of more than 99%.

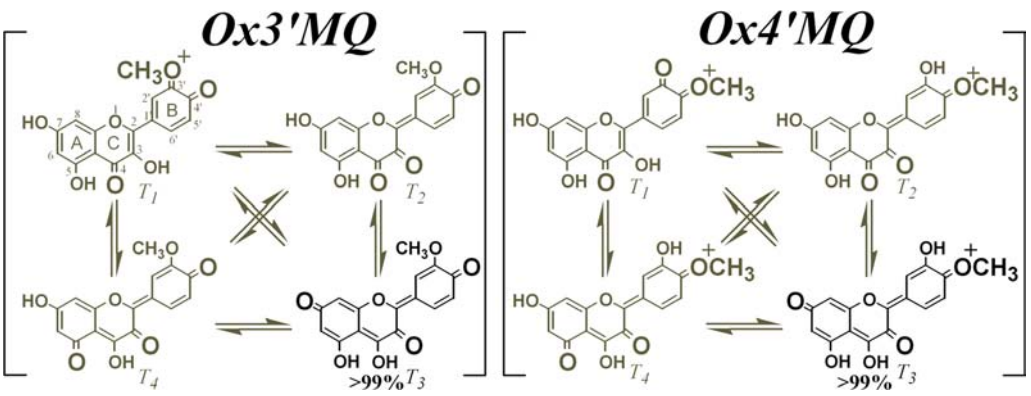

Figure 4. Tautomers of oxidized **3′MQ** (**ox3′MQ**) and oxidized **4′MQ** (**ox4′MQ**). For **ox3′MQ**, T_3 is the most favorable tautomer because the carbonyl groups are at maximum distance from each other. In **ox4′MQ**, all the possible tautomers are positively charged. Also for **ox4′MQ**, T_3 is the most favorable. Because of its positive charge the chemical nature of **ox4′MQ** is much "harder" than that of **ox3′MQ**. Quantum molecular calculations indicate that the preferred tautomer of both compounds has a relative abundance of more than 99%.

DISCUSSION

In the present study, the effect of O-methylation of **Q** on the competition between ascorbate and thiols, with GSH as thiol, for the oxidized flavonoid is determined. The oxidized flavonols have four tautomeric forms, denoted as T_1, T_2, T_3, and T_4 in the present study. In T_1 the carbonyls are located at the 4, 3′, and 4′ positions; in T_2 they are at the 3, 4, and 4′ positions; in T_3 they are at the 3, 7, and 4′ positions; in T_4 they are at the 3, 5, and 4′ positions.

Q vs 3MQ. It appears that the CAT of **3MQ** (1.77) is substantially higher than that of **Q** (0.04). For **oxQ**, T_3 appeared to be the most abundant, which has also been reported previously.[10] In the case of **ox3MQ**, the electronically uncharged T_1 form is energetically the most favorable one because the other tautomers have an unfavorable positive charge. Therefore **ox3MQ** will almost exclusively (>99%) be in the T_1 form. The difference in structure between **oxQ** (T_3) and **ox3MQ** (T_1) forms the basis of their difference in CAT, which can be explained by applying Pearson's HSAB concept.[11] The gist of this theory is that "soft" electrophiles react faster with "soft" nucleophiles, whereas "hard" electrophiles react faster with "hard" nucleophiles. "Hard" electrophiles are chemical species that are small, highly polarized, and have a high oxidation state.[11,12] The difference in "hardness" between the most abundant tautomer of **OxQ** and **ox3MQ** lies mainly in their polarity. The carbonyl groups represent the most polarized moiety, and a close proximity of these groups will make the oxidized compound "hard".

In T_3, which is the most abundant tautomer of **oxQ**, the distance between the carbonyl groups is at its maximum, making it the "softest" electrophile of the series. Thiols such as GSH are known to be "softer" than ascorbate.[10] The HSAB concept dictates that the "soft" electrophile, **oxQ**, should react faster with the "softer" GSH than with ascorbate, explaining the relatively low CAT of **Q**.

In T_1, which is the most abundant tautomer of **ox3MQ**, the two carbonyl groups in the B-ring are adjacent, making the molecule relatively "harder" than **oxQ**. In contrast to **oxQ**, the relatively "harder" **ox3MQ** prefers the "hard" ascorbate over the "soft" GSH, explaining the higher CAT of **ox3MQ**. This exemplifies the correlation of the CAT with the "hardness" of the oxidized flavonoid; the "harder" the oxidized product, the higher the CAT.

3MQ vs 3RuQ (Rutin). **Ox3MQ** and **ox3RuQ** are substituted at the same position, and T_1 is the most preferred tautomer for both compounds. However, the CAT of **3RuQ** (2.90) is higher than that of **3MQ** (1.77). Quantum molecular calculations showed that the bulky rutinosyl group at the 3-O position in the case of **ox3RuQ** induces a torsion angle (dihedral) of 29° between the plane of the AC-ring and that of

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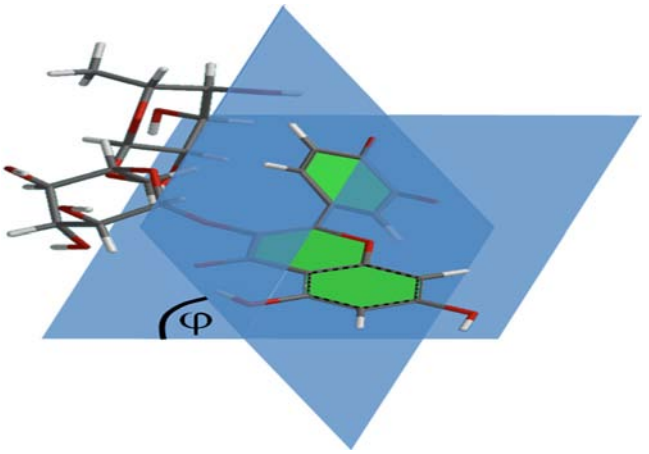
Table 2. Most Abundant Tautomer of the Oxidized Flavonoids, Their LUMOs, and Their Dihedrals (φ)[a]



compd	most abundant tautomer	LUMO (kJ/mol)	φ (degrees)
OxQ	T_3	−112.14	0.0
Ox5MQ	T_3	−98.35	0.0
Ox7MQ	T_4	−74.00	0.0
Ox3′MQ	T_3	−49.45	0.0
Ox3MQ	T_1	−15.74	13
Ox3,7MQ	T_1	−11.24	13
Ox3RuQ	T_1	−24.30	29
Ox4′MQ	T_3*	21.33	0.0

[a]The dihedral angles are the angles between the plane of the AC-ring and that of the B-ring. The tautomeric forms are denoted as T1, T2, T3, and T4. In T1 the carbonyls are located at the 4, 3′, and 4′ positions; in T2 they are at the 3, 4, and 4′ positions; in T3 they are at the 3, 7, and 4′ positions; in T4 they are at the 3, 5, and 4′ positions. The asterisk (*) indicates that the tautomer contains a positive charge.

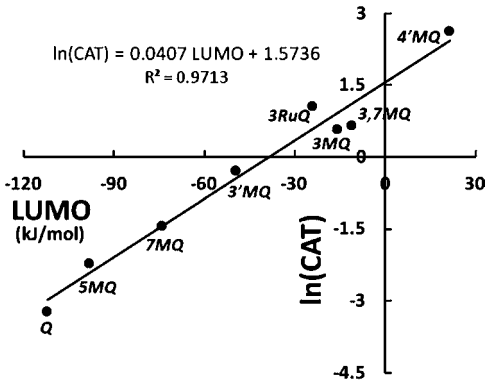


Figure 5. Correlation of the energy of the LUMO of the most abundant tautomer of **oxQ** and its derivatives with the competition between ascorbate and thiols (CAT) for these oxidized flavonoids.

the B-ring in its most preferred tautomer (Table 2). The methyl group in **ox3MQ** induces a dihedral of only 13°, whereas in **oxQ** the dihedral is 0.0°. A higher dihedral reduces the interaction between the π-system of the AC and that of the B-ring due to less $\pi-\pi$ overlap, and thus it further concentrates the polarity on the B-ring. This means that **ox3RuQ** will be more polarized than **ox3MQ**, making **ox3RuQ** a "harder" electrophile that is reflected in the higher CAT of **3RuQ**. Actually, **oxQ**'s complete planar structure indicates a maximal interaction between the AC- and the B-ring. This strong $\pi-\pi$ interaction, over the entire molecule, in **oxQ** forms the backbone of its "soft" character.

3′MQ vs 4′MQ. The difference in CAT between **3′MQ** (0.75) and **4′MQ** (14.5) is remarkably high. Similarly to **oxQ**, T_3 is also the preferred form for **ox3′MQ** that has a dihedral of practically zero. This indicates that the "softness" of **ox3′MQ** is close to that of **oxQ**, explaining their relatively similar CATs.

In the derivatives discussed so far, their most abundant tautomer had no positive charge and the most polarized groups were the carbonyl moieties. In the case of **ox4′MQ**, all the possible tautomers have a positive charge located on the 4′ position as depicted in Figure 4. This positively charged moiety is much more polarized than the carbonyl groups. The presence of a moiety with a positive charge renders **ox4′MQ** to be the "hardest" electrophile in our series. This explains why **4′MQ** has the highest CAT in our series. The most preferred tautomer of **ox4′MQ** is also T_3 with a dihedral of practically zero. This indicates that the "hard" character of **ox4′MQ** is not due to a distortion of the planar backbone of the molecule.

5MQ and 7MQ. The most abundant tautomer of **ox5MQ** (T_3) and that that of **ox7MQ** (T_4) have no positive charge, and the distance between the carbonyl groups is relatively large. This indicates that the difference in "softness" of **ox5MQ** and **ox7MQ** with **oxQ** is relatively small, explaining their comparable CATs (Table 1).

3,7MQ. We have also tested a dimethylated **Q** derivative, namely **3,7MQ**. The CAT and the dihedral of this compound are similar to that of **3MQ**, which confirms that the dihedral plays a pivotal role and emphasizes the importance of the $\pi-\pi$ interaction of the AC- with the B-ring.

Correlation between the LUMO and the CAT. Chemical reactions are generally governed by the interaction between the frontier orbitals, more specifically, the interaction between the HOMO (highest occupied molecular orbital) of the nucleophile and the LUMO of the electrophile. "Hard" electrophiles are known to have relatively higher LUMO energies than "soft" electrophiles.[12] Because all reactants in the oxidation assay were the same, except for the oxidized flavonoids, we focused on the LUMO energies of the most abundant tautomer of the oxidized flavonoids. The LUMOs of the oxidized flavonoids show a high correlation with their CATs as depicted in Figure 5. This correlation is in line with the theory that a "harder" molecule has a higher LUMO energy, which is reflected in lower thiol reactivity and a higher CAT.

Q Derivatives That Would Preferentially Interact with Ascorbate over Thiols. To decrease thiol toxicity, the **Q** derivative has to have a higher preference for ascorbate than for thiols, i.e. it has to have a high CAT. Methylation at the 5 and 7 position hardly affects the CAT. Methylation at the 3′O or the 3O position leads to a relatively small increase in the CAT. This effect is enhanced when a relatively large substituent is placed at the 3 position, since this greatly distorts the planar structure of **Q**. By far, the greatest effect was found when the 4′ position was substituted. In fact, methylation at the 4′ position decreases the thiol reactivity of **Q** more than 350 times.

It appears that for a high CAT, methylation at 4′O position of **Q** is the most effective. In general, O-methylation will reduce the antioxidant effect of quercetin. Nevertheless, **4′MQ** still possesses significant antioxidant activity.[6,13] Moreover, **ox4′MQ** will be regenerated by ascorbate in the antioxidant network to **4′MQ** that will boost its potency. Additionally, it has been demonstrated that the cellular uptake of **4′MQ** is superior to that of **Q**.[7,8] Our data indicate that **4′MQ** would be

less toxic than **Q**. Spencer et al.[7] have shown that this is indeed the case.

It is becoming increasingly more evident that metabolites of flavonoids are partly, or even primarily, responsible for the health benefits. In particular, the methylated flavonoids are of interest.[8,14] Interestingly, **4′MQ** is formed in vivo by catechol O-methyl transferase (COMT) as one of the metabolites of **Q**.[15] The present study indicates that the disadvantageous thiol toxicity of **Q** is diminished in **4′MQ**. The endogenously formed **4′MQ** might be a physiological way to safely benefit from the antioxidant potential of quercetin in vivo.

■ ASSOCIATED CONTENT

Supporting Information

Figure of the delocalization of the LUMO of **oxQ** (T_3) and **ox4′MQ** (T_3), scheme of the proposed reaction mechanism of **oxyQ** with water, and a table containing the ^{1}H NMR signal assignment of the oxidation product in DMSO-d_6. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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