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Unveiling the chemistry behind bromination of quercetin: the 'violet chromogen'

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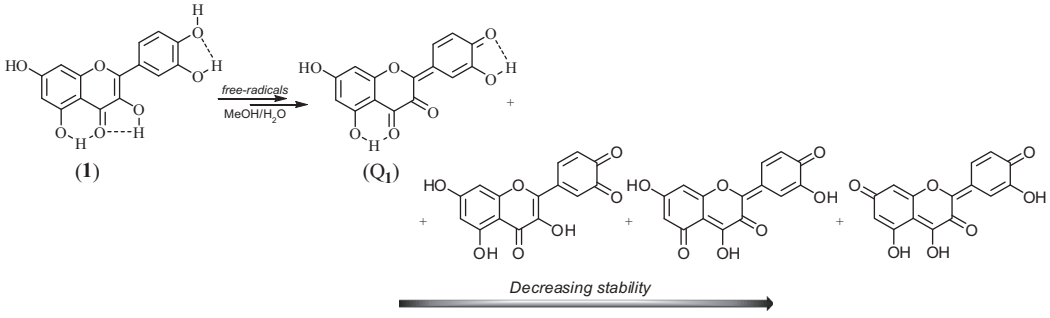
ABSTRACT

Bromination of quercetin with *N*-bromosuccinimide in neutral aqueous methanol occurs surprisingly in the *electron-deficient* A-ring only. Deprotonation of the acidic 7-OH is a major driver of this regioselective reaction. The increase of electron density makes in fact the quercetin anion suitable for an electrophilic attack by bromine at positions 8 and 6. Several pieces of evidence (NMR spectra and H/D exchange) are presented to substantiate the mechanism advanced. Bromoquinones/quinomethides produced in excess of *N*-bromosuccinimide are responsible for the formation of a stable 'violet chromogen'.

Quercetin (**1**) is a polyphenol belonging to the class of 'flavonoids' which are widely distributed in the plant kingdom and consequently in our daily diet.[1,2] Quercetin has recognized biological properties[3–5] and—as most phenols[6]—is able to slow down the process of oxidation of organic matter[7] caused by dioxygen $^3O_2$ (peroxidation). This beneficial antioxidant property is due to the ability that quercetin has to chelate transition-metal ions and to quench peroxyl radicals ROO·.[6,7] The interest in the chemistry and biology of quercetin is therefore notable, and in the last decades has shown no decline.[8]

The oxidation chemistry of quercetin has long been investigated.[9–11] The two-electron oxidation yields quinone/quinomethide compounds (Eq. (1)) that are intensely colored in purple ($\lambda_{max} \sim 525$ nm, in 80% by volume methanol/water).[9] Density functional theory (DFT) calculations[12] show that the quinomethide Q_1 is more stable—and thus more abundant in solution—than the other three possible tautomers (Eq. (1)).



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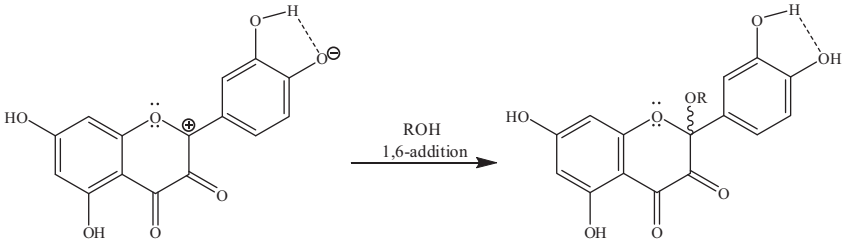
In protic solvents, the survival of the above quinone/quinomethide species (reaction 1) is however very limited. In fact, in 80% methanol/water (v/v) the half-life of Q_1 is 2.5 s only.[9] Q_1 can be regarded as a resonance-stabilized benzylic carbocation which readily undergoes a proton-assisted (Michael-type) nucleophilic addition of solvent (ROH) at position 2 (and 3) (Eq. (2)).[13,14] Discoloration of the solution follows this reaction as a consequence of the interruption of the conjugation between the rings B and A+C (Eq. (2)). At room temperature, the reaction in 80% methanol/water (v/v) is over, that is the purple color disappears, in a few tens of seconds.[9]

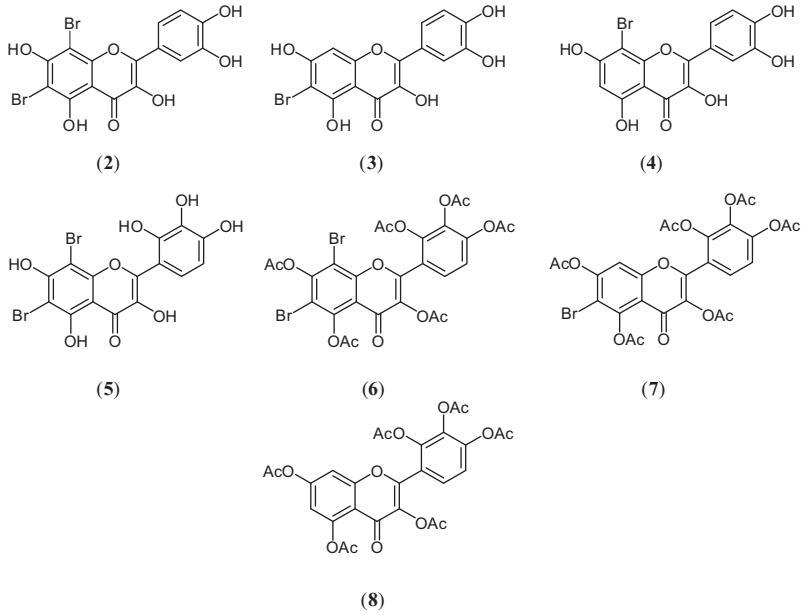


(2)

The aforementioned instability of Q_1 in protic solvents, however, seems to contrast with a report of 1992 in which the authors affirm that a methanolic solution of quercetin upon treatment with a neutral aqueous solution of *N*-bromosuccinimide (NBS) produced instantaneously an intense 'violet chromogen' ($\lambda_{max} \sim 510$ nm) which was stable for at least 15 min.[15] Later, the procedure was slightly modified and it was reported that the violet color persisted without decaying for more than one hour.[16,17] Interestingly, solutions of this oxidized quercetin reagent were used to titrate ascorbic acid and several antibiotics (cefoperazone sodium, cefazolin sodium, and ceftriaxone sodium) with a simple and accurate spectrophotometric test.[16,17]

The authors of these works attributed the violet color to the formation of quercetin quinones/quinomethides, in particular to Q_1.[15,16] This hypothesis has been reconfirmed until recently[16] after about 20 years from the first observation. Our data, however, do not support this conclusion because Q_1 disappears very quickly in methanol/water mixtures (see above). *What is (are) therefore the compound(s) responsible for this persistent and intense violet color*? While answering this question we chanced upon a few derivatives of quercetin (bromoquercetins and 2′-hydroxy-6,8-dibromoquercetin, see Scheme 1) worthy of being mentioned because we discovered they possess singular properties that will be reported in a forthcoming Letter. Although a few of these compounds are already known,[18] the syntheses we now report (see Supplementary data) are particularly simple and environmentally-friendly deserving therefore consideration.

First, we verified that upon treatment of a methanol solution of quercetin with aqueous NBS in a mole ratio of 1:4, respectively, the solution became immediately violet and the color persisted for



Scheme 1. Quercetin bromoderivatives obtained by treating a methanol solution of quercetin with aqueous NBS followed by reduction with $Na_2S_2O_4$ at room temperature. The acetates were obtained by treating the reaction mixtures with acetic anhydride/pyridine.

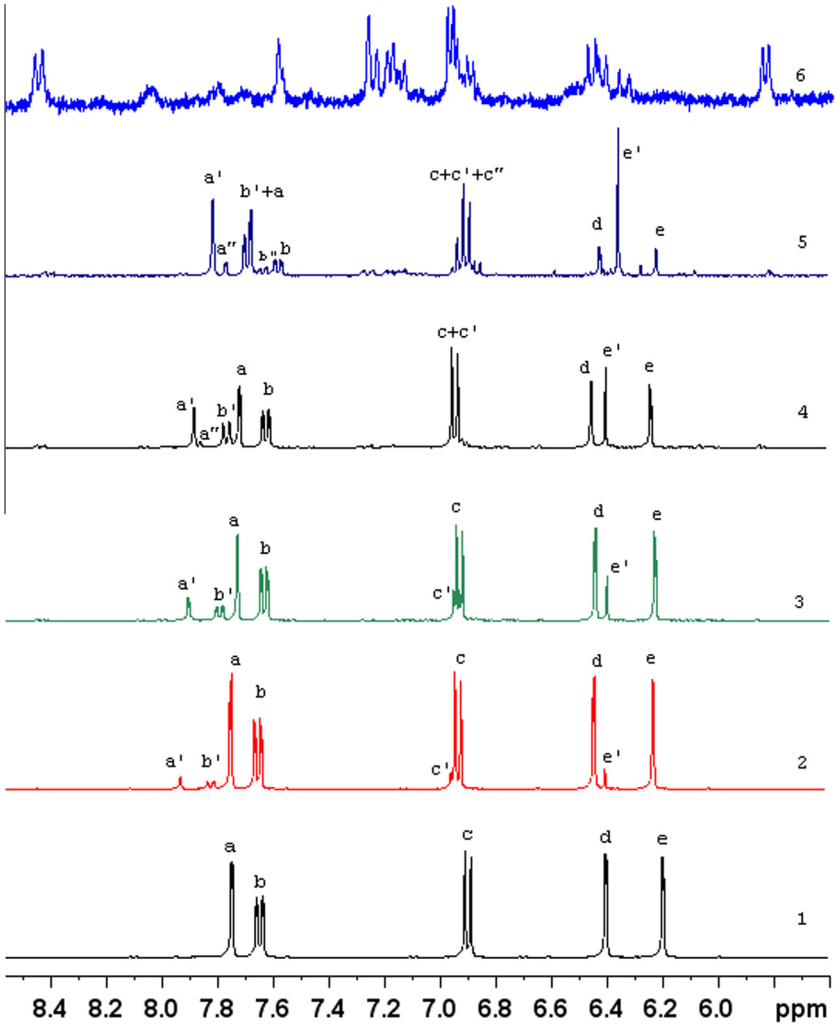
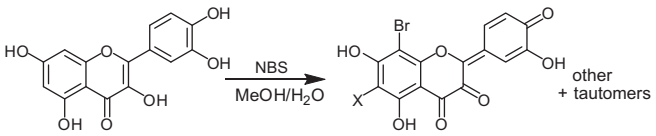


Figure 1. (1) NMR spectrum of quercetin **1** (19.2 mM) in methanol-d_4; the peaks *a*, *b*, and *c* correspond to the B-ring protons 2′, 6′, and 5′, respectively; *d* and *e* correspond to the A-ring protons 8 and 6, respectively. (2) Addition of a D_2O-solution of NBS (91.2 mM) at a mole ratio NBS:**1** of 0.5; the peaks *a*′, *b*′, *c*′, and *e*′ correspond to the protons 2′, 6′, 5′, and 6, respectively, of 8-bromoquercetin **4**. (3) Same as for (2) with NBS:**1** of 0.85. (4) NBS:**1** of 1.6. (5) NBS:**1** of 2.8. (6) NBS:**1** of 3.8.

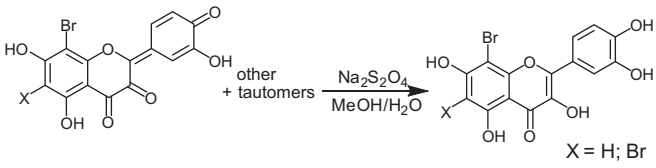
hours. The presence of water was essential since in pure methanol there was no formation of the chromogen. The reaction in deuterated methanol+D_2O had the same outcome but the ^{1}H NMR spectrum of the solution—recorded immediately after mixing the chemicals—was difficult to interpret because it contained six *weak* and partially unresolved groups of signals scattered in the chemical shift range 5.8–8.5 ppm (see Fig. 1). The intensity of the signals decreased in time and after about 15 min the signals disappeared from the NMR spectra. We believe this peculiar behavior of the proton signals was due to free-radicals in solution which broadened the signals beyond detection (paramagnetic relaxation enhancement).[19,20]

In previous experiments on the kinetics of oxidation of quercetin with **dpph**·,[9] it was observed that the quinomethide Q_1 could be reconverted to quercetin (in non-protic solvents, e.g., CH_2Cl_2) by treatment with excess ascorbic acid palmitate at room temperature (see the Supporting information of Ref. 9). The UV–vis spectra showed a complete and 'clean' formation of quercetin and disappearance of the colored quinone ($\lambda_{max} \approx 525$ nm) with the maintenance of an isosbestic point at about 392 nm. We therefore treated a methanol/water solution of the violet chromogen—obtained as described above—with ascorbic acid or (better) sodium dithionite $Na_2S_2O_4$ which caused a quick discoloration of the solution and the formation of a precipitate. The NMR and MS spectra revealed that the precipitate was 6,8-dibromoquercetin **2** (97% pure). The final yield of this one-pot synthesis was about 70%. By changing the pH of the solution we selectively obtained the 6-bromoquercetin **3** (adding NaOH) and the 8-bromoquercetin **4** (adding HCl) in mixture with variable quantities of **2**. Upon changing the solvent from aqueous methanol to aqueous acetone the reaction yielded reproducible and significant quantities of a new bromoflavonoid **5** together with 6,8-dibromoquercetin **2** in a 1:1 ratio (see Supplementary data). The precipitate, obtained after treatment with $Na_2S_2O_4$, was acetylated with acetic anhydride/pyridine in CH_2Cl_2 at reflux and chromatographed over silica gel to yield the acetylated form **6** (final yield 35%) and small quantities of **7** and **8** (see Scheme 1).[21]

The foregoing data suggest that in neutral or weekly acidic solutions the 'violet chromogen' is principally constituted of a mixture of 6,8-dibromo- and 8-bromoquinones/quinomethides in tautomeric equilibrium with their respective isomers (Eq. (3)). These are easily reduced by $Na_2S_2O_4$ to brominated quercetin (Eq. (4)). The ^{1}H NMR spectrum of the 'violet chromogen', although weak (see above), is compatible with the structure of the above compounds.



(3)



(4)

The presence of bromine in the A-ring makes the quinones/quinomethides survive in protic solvents almost indefinitely. The same is not true for the non-brominated counterparts produced in (Eq. (1)) which react quickly with protic solvents (Eq. (2)).[9] It is likely that bromine destabilizes the benzylic carbocation mentioned above (Eq. (2)) by field effects. In other words, we think that the zwitterionic forms **I** and **II** are less important than the covalent form **III** in the resonance hybrid (see Scheme 2) of the brominated quinones/quinomethides. This might therefore make the addition of ROH more difficult.

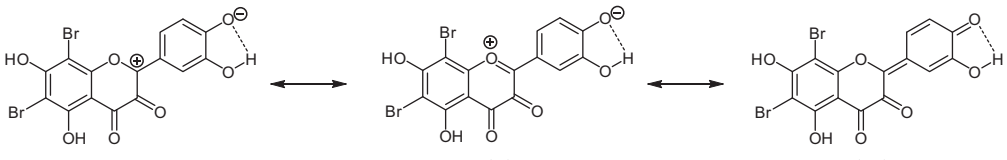
Further, it is worth noting that under our experimental conditions bromination of quercetin occurred in the A-ring only—although the apparent target of this electrophilic aromatic substitution should be the B-ring instead. The catechol ring of quercetin is, in fact, electron-richer than the A-ring which suffers the withdrawing field and mesomeric effects of the carbonyl group at the 4-position.[9–11,22,23]

To explain the regioselectivity of NBS, we had initially advanced the hypothesis that quercetin was rapidly oxidized to the quinone form Q_1 by NBS rather than being brominated. Oxidation of quercetin in aqueous media is expected to be fast because it can involve an electron-transfer process from quercetin anions (vide infra).[9] The A-ring of *oxidized* quercetin (Q_1) is electron richer than the B-ring and could therefore be the accessible site for bromination. However, this explanation demanded another restrictive requirement. Bromination of Q_1 had to occur faster than its decay by solvent addition (Eq. (2)). The fact that reaction 2 is fast (vide supra) and that Q_1 is a *deactivated* substrate for electrophilic reactions makes this requirement difficult to meet.

In fact, the NMR spectra of **1** added with scalar amounts of NBS depicted a different scenario, see Figure 1. Addition of increasing amounts of NBS in D_2O to a methanol-d_4 solution of **1** caused exclusively the formation of monobromoquercetin **4** and of dibromoquercetin **2** (at higher ratios NBS:**1**, see Fig. 1) without involving Q_1 or any oxidized forms of bromoquercetins. In other words, *bromination occurred directly onto the A-ring of quercetin*. Quinones were formed after bromination when the molar ratio NBS:**1** exceeded 3. This finding forced us to abandon the explanation given above and to assess the effects of the hydroxyls of the A-ring on the reaction.

The ^{1}H NMR spectrum of quercetin in acetone-d_6 displays five sharp peaks attributed to the five OHs by long-range experiments, see Figure 2. Addition of H_2O (or traces of NaOD) caused a large broadening or disappearance (H_2O >8% by volume) of the signal of the 7-OH (δ 9.75), see Figure 2. This suggests that the acidity of the 7-OH is higher than that of the other OHs.[24] Hence, the 7-OH must be the primary site of deprotonation of quercetin. The behavior of the C-7, H-6, and H-8 NMR signals after the addition of NaOD reinforces this conclusion.[25] Indeed, our findings are in agreement with the results of several other investigations which all support the conclusion that the 7-OH is the most acidic hydroxyl in quercetin.[26] In particular, Litwinienko and co-workers[26] have recently estimated that the pK_a of the 7-OH is in the range 7.5–8.5. They have also analyzed the implications that this relatively large acidity has in the reactions of quercetin with radicals in ionizing solvents.

On the basis of the pK_a range reported above, it is plausible that the *anion* of quercetin, as for other phenols,[27] may well be the *true* substrate of bromination, see Scheme 3. That is, it is most likely that



Scheme 2. Canonical resonance structures of dibromoquercetin quinomethide.

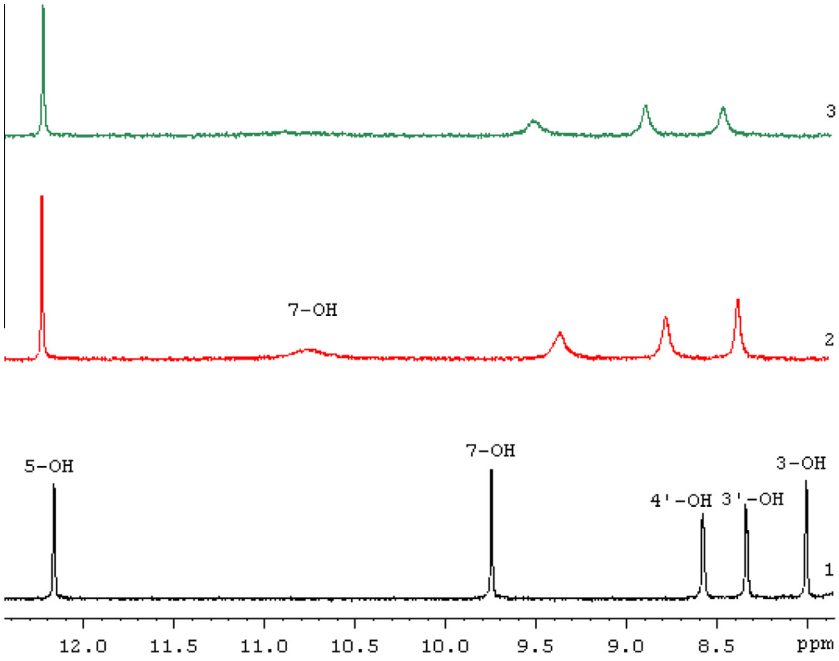
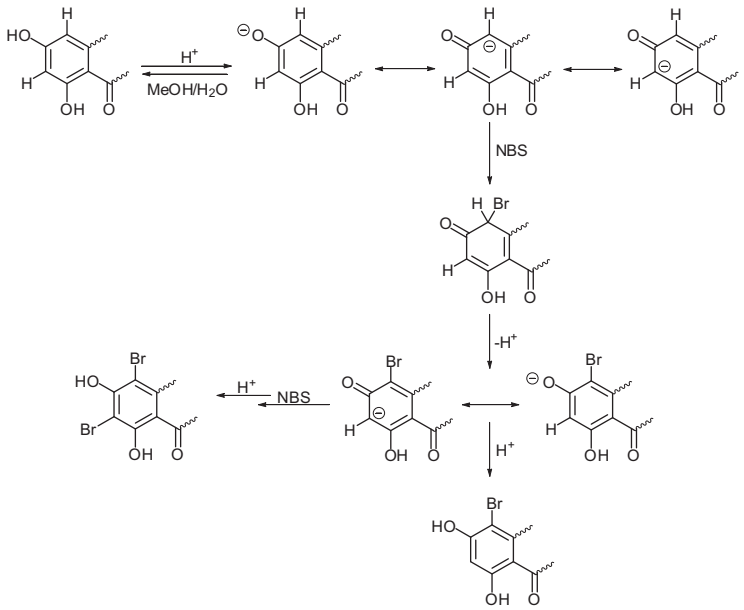


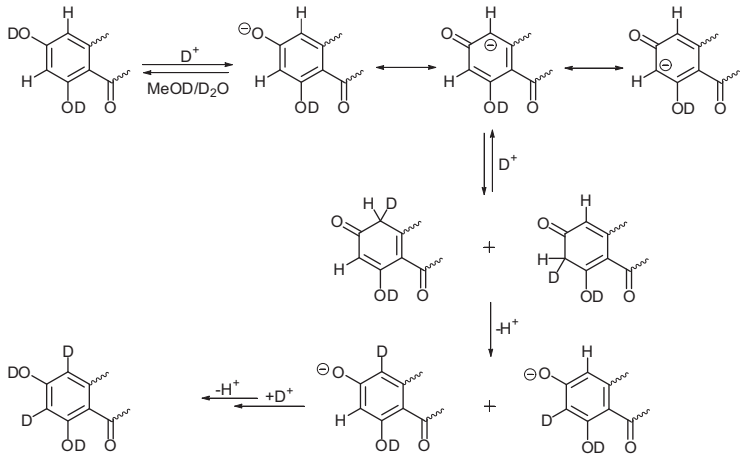
Figure 2. (1) ^{1}H NMR of quercetin in acetone-d_6 showing the region of the OH resonances. The assignment was done by HMBC experiments. (2) Same as above after the addition of 4% (v/v) H_2O. The hump at about 10.7 ppm is due to the 7-OH which is the most acidic site in quercetin, see text. (3) After addition of 8% (v/v) H_2O, see text.



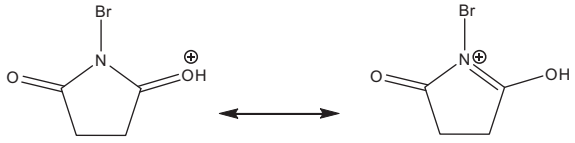
Scheme 3. Mechanism of bromination of quercetin with NBS in methanol/water, see text.

the A-ring of quercetin is activated toward electrophilic substitutions by ionizing solvents through deprotonation of the 7-OH. This conclusion is further supported by some additional observations we came across during the NMR experiments. Quercetin displayed a singular hydrogen/deuterium exchange at the positions 6 and 8 that was apparently driven by the ionization of the 7-OH. We observed that the NMR signals of the protons 8 and 6 *slowly*[28] disappeared when a methanol-d_4/D_2O solution of quercetin was treated with diluted NaOD or DCl (the details will be reported in a forthcoming Letter), see Scheme 4. A complete isotope exchange took place in our conditions also because the bond enthalpy of C-D is greater than that with the lighter isotope, C–H.[29]

The involvement of the quercetin anion in the bromination mechanism represented in Scheme 3 accounts for a few details of the reaction that otherwise would be difficult to explain. If the reactive species was the neutral quercetin, the rapid double bromination of the A-ring, for instance, would hardly be explained because the monobrominated species **3** and **4** would be further deactivated as substrates of electrophilic reactions. Actually, once the first Br atom enters the ring the second bromination step can be further accelerated by the increased acidity of the 7-OH in the monobromoquercetins.[30] On the other hand, addition of diluted HCl did not inhibit the bromination reaction, although to some extent it certainly depressed the ionization of the 7-OH. This might



Scheme 4. Mechanism of H/D exchange in the A-ring of quercetin in methanol-d_4/D_2O containing DCl, see text.



Scheme 5. Canonical resonance structures of protonated NBS.

indicate that the acidity of the 7-OH can be comparatively larger than previously reported[26] but we cannot really exclude that under (strongly) acidic conditions bromination may occur without involvement of the quercetin anion.

Yet, addition of HCl may also induce an increase in the reactivity of NBS, that is, in the electrophilicity of its Br atom, through protonation of one carbonyl group, see Scheme 5. It is known in fact that highly deactivated aromatic compounds can indeed be smoothly monobrominated by treatment with NBS in concentrated H_2SO_4 medium.[31]

In conclusion, bromination of quercetin with NBS in methanol/water occurs rapidly in the A-ring only and yields—through a simple one-pot and environmentally-friendly synthesis—various interesting brominated derivatives by changing the pH or the organic solvent. We have advanced a reasonable mechanism which is based on the prior ionization of the 7-OH and on the successive electrophilic attack of Br at the electron-rich positions 8 and 6 of the quercetin anion. A singular H/D exchange at the position 8 and (more slowly) at the position 6 of quercetin occurs reasonably with a similar mechanism. All these findings fully confirm the comparatively high acidity of the 7-OH relative to the other OHs. Excess of NBS over quercetin produces intensely colored and surprisingly stable bromoquinones/bromoquinomethides. Finally, the mechanisms described yield bromoderivatives that can be useful intermediates for the regioselective functionalization of quercetin and other flavonoids.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2014.01.081.

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25. See the Supporting information of Ref. 9 for more details. In brief, the resonances at ca. 6.45 and 6.24 due to H-8 and H-6 (in 80% methanol-d_4/D_2O), respectively, after addition of 0.5 equiv of NaOD shifted upfield by ca. 0.2 ppm. This large shift is most likely due to the fact that the negative charge in the quercetin monoanion is essentially delocalized in the A-ring, therefore suggesting that ionization takes place at the 7-OH. Further, the C-7 signal, upon addition of NaOD, shifted downfield toward the carbonyl (C-4) signal by more than 5 ppm (163.68–168.74 ppm). Again, this confirms that ionization occurs at 7-OH because the C7-O^- anion has a strong C=O character in the resonance hybrid of the anion, see Ref. 9.
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