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Solvent-dependent Release of Bromine from Bromoquercetins

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Abstract: Quercetin, 6-bromoquercetin (**3**) and 8-bromoquercetin (**4**) undergo H/D exchange at 6- and 8-positions, in acetone-d_6 and methanol-d_4, catalyzed by acids and bases. The base-catalyzed process is faster, and in acetone-d_6 the half-lives of H-8 and H-6 are 56.5 and 48.6 hours, respectively. A high regioselectivity at the position 8 of quercetin is displayed under acid-catalysis in both solvents but in methanol-d_4 a significantly high regioselectivity is retained even under base-catalysis. On the other hand, 6,8-dibromoquercetin (**2**), **4** and 6,8-dibromo-2'-hydroxyquercetin (**5**) manifest the ability of exchanging *bromine* with hydrogen (or deuterium) under acid-catalysis in *acetone* and other enolizable ketones (e.g. methyl ethyl ketone, acetylacetone and isophorone). These bromophenols release bromine from their 8-position only, in a *slow* bromination process that likely involves their protonated form (arenium ion **I**) as brominating agent and the enol of the above ketones as Br-acceptor. The arenium ion **I** of these bromophenols is expected to be a powerful electrophile and its formation is most likely to be rate-determining.

Keywords: Quercetin; Bromoquercetin; Enol; Bromination; Acetone; H/D exchange; Debromination.

We have recently published a Letter in this Journal on the synthesis and mechanism of formation of bromoderivatives **2 – 5** of quercetin (**1**), see Scheme 1.[1] The 2e-oxidation of these compounds produces bromoquinones/quinomethides intensely colored. These bromoquinones/quinomethides are remarkably stable in protic solvents (e.g. methanol and water) where the acid catalyzed 1,6-addition of solvent (which would cause discoloration of the solution) occurs extremely slowly, if at all.[1] In neutral aqueous methanol, quercetin is brominated by *N*-bromosuccinimide at positions 6 and 8 of the electron-deficient A-ring probably because this electrophilic substitution occurs in the *anion* of quercetin from the 7-OH.[1]

Bromoquercetins **2**, **4** and **5** (see Scheme 1) synthesized in these experiments showed the property of exchanging their Br atoms with H or D. This exchange occurred in acidified acetone, and more in general in ketones with α-hydrogens, while in methanol, water/methanol mixtures, acetone/methanol mixtures and DMSO it did not occur at all, *vide infra*. Complete Br/H exchange was slow and, at room temperature, required several days. However, at ca. 60 °C the exchange took place within a few hours. During these experiments we also observed that the aromatic hydrogens of the A-ring of quercetin and of mono-bromoquercetins **3** and **4** exchanged with deuterium at detectable rates.

<Scheme 1 about here >

Generally, removal of halogen from aromatics can be accomplished by reducing agents, Friedel-Crafts catalysts, and by photochemical and electrochemical reduction.[2] The experimental conditions of these methods are usually not mild. Chlorinated benzenes and naphthalenes, for instance, can be hydrodehalogenated by a H-atom donating solvent such as 9,10-dihydroanthracene at temperatures between 530 and 630 K.[3] In our case, debromination of **2**, **4** and **5** occurred instead under very mild conditions, the process requiring HCl as a catalyst and an *appropriate* solvent only. We eventually discovered that the apparent Br/H "exchange" was actually a consequence of bromination of acetone. The reagent involved in this process was the "elusive" enol form of

acetone. At any rate, the reaction seemed to be induced by some distinctive features of these polyphenols (*vide infra*) that were not apparent because 2,6-dibromophenol, for instance, did not show any tendency to release bromine. An acetone solution of this phenol added with HCl and left at room temperature for one month did not show any visible change in its ^{1}H NMR spectrum. The C-X bond (X = H, D, Br) at 8 position and more moderately at 6 position of quercetin are instead astonishingly "loose" probably because of tautomeric equilibria (*vide infra*) established in solution. A similar behavior was also reported for 6- and 8-glutathionylquercetin (GSQ) adducts which left in solution gave rise to isomerization up to an equilibrium composition, 6-GSQ ⇆ 8-GSQ, of about 55:45.[4] On the other hand, cysteine adducts of quercetin were reported not to isomerize perhaps as a consequence of a hydrogen bond between cysteine NH_2 and one quercetin O atom.[5] The behavior of **1** – **5**, reported in the present Letter, is therefore worthy of note and to our knowledge rather uncommon.

H/D Exchange in Quercetin

When a 10 mM solution of quercetin in methanol-d_4, treated with 6 mM DCl and left aside for 10 days, was scanned with the NMR spectrometer, we discovered that the signals of H-6 and H-8 at 6.24 and 6.46 ppm, respectively, had a lower intensity relative to the B-ring protons. Precisely, H-8 integrated to 0.63 instead of 1.0 and H-6 to 0.95, see Figure 1. After 12 more days, the integration of the H-8 peak reduced to 0.37 while the H-6's to about 0.93. In these NMR experiments the recycle delay (d1) was set to 20 seconds (90° pulse), a sufficiently long time to guarantee an accurate integration of the peaks since the longest relaxation time T_1 of the proton signals was 2.50 seconds (H-6 proton).[6]

<Figure 1 about here>

The above data clearly indicate that the H-atoms at C-8 and C-6 positions exchanged with deuterium. The bond enthalpy of C-H is smaller than that of C-D and thus the replacement of H with D is thermodynamically favored.[7] The rate of H/D exchange at C-8 increased with increasing concentrations of DCl with a reaction order of ~ 1.[8] From the above data, the first-order[8] rate constant for the slow disappearance of H-8 was estimated to be 5.56×10^{-7} s^{-1} at [DCl] ≈ 6 mM. The half-life $\tau_{1/2}$ of this proton under the above conditions was therefore 1.24×10^{6} s (corresponding to 345 h), see Table 1. The catalysis of DCl was essential since in pure methanol-d_4 the process was very slow and the changes in the peak area too small to be evaluated with accuracy.

The NMR spectra showed that the acid-catalyzed H/D exchange proceeded with high regioselectivity since the exchange did not, *essentially*, involve any B-ring protons[9] and H-6 underwent exchange so slowly that we prevent ourselves from any evaluation, see Table 1 and Figure 1. Similar results were also observed in acetone-d_6 added with 6 mM DCl. Again, the H/D exchange was highly regioselective at C-8 position but it was 4-fold slower than in methanol-d_4 the rate constant being 1.39×10^{-7} s^{-1} and $\tau_{1/2}$ ≈ 1380 h, see Table 1.

H/D exchange in the aromatic rings of quercetin was previously reported. Madhusudanan and co-workers[10] reported in 1984 that aromatic hydrogens of several flavonoids underwent exchange with deuterium under gas-phase D_2O chemical ionization conditions. Andersen and co-workers[11] in 2007 and then Faizi and co-workers[12] in 2010 both reported similar H/D exchanges in solution. The authors observed that CF_3COOD in methanol-d_4[11] or in DMSO-d_6[12] catalyzed regioselectively H/D exchange at the C-8 position of quercetin[12] and of a quercetin 3-*O*-glycoside.[11,13] In these papers, however, there is no mention of the possible effects that a base can have on the H/D exchange. We therefore undertook a kinetic study using this time NaOD as a catalyst.[14] We discovered that the rate of H/D exchange at C-8 in methanol-d_4 containing 6 mM NaOD increased by 3-fold relative to the acid-catalyzed process (see Figure 2 and Table 1), the observed rate constant being 1.67×10^{-6} s^{-1}. Furthermore, H-6 underwent, this time, exchange with deuterium at a detectable rate, $k_{obs} = 2.78\times10^{-7}$ s^{-1}. The rate increase was even higher in acetone-d_6

<Figure 2 about here>

where addition of 6 mM NaOD caused a significant acceleration of the exchange process *both* at C-8 and at C-6, k_{obs} = 3.39×10^{-6} and 3.94×10^{-6} s^{-1}, respectively, see Figure 3. The regioselectivity observed in the acid-catalyzed exchange, therefore, levelled off (see Table 1). Interestingly, addition of 20% D_2O to acetone-d_6 caused a large prevalence of the exchange at C-6 over that of C-8. In methanol-d_4, however, a high regioselectivity at C-8 was retained, see Table 1.

<Figure 3 about here>

The H/D exchange that quercetin undergoes in solution can be ascribed to keto-phenol tautomerism. The process may be visualized as proceeding via an arenium ion intermediate (acid catalysis, see Scheme 2) or via a cyclohexadienone intermediate (base catalysis, see Scheme 2).[15] The preferential electrophilic attack at positions 6 and 8 parallels the largely up-field shifted ^{13}C NMR δ values of C-8 and C-6 (93.5 and 98.3 ppm, respectively, in acetone-d_6) relative to the C-atoms of the B-ring (δ 115 – 148 ppm, in acetone-d_6). These up-field chemical shifts reflect a large π-electron density that justifies the preferential electrophilic attack on these two carbon atoms.

<Scheme 2 about here>

Br/H "exchange" in quercetin derivatives 2, 4 and 5

Treatment of an *acetone* solution of 14 mM 6,8-dibromoquercetin **2** with 200 mM HCl led to its conversion to 6-bromoquercetin **3**.[16] At room temperature, the process required several days but heating the solution at reflux, complete conversion occurred within some hours. The loss of bromine from the 8 position of **2** was monitored by NMR spectroscopy observing first the

appearance and then the growth of the H-8 signal of **3**, see Figure 4. This reaction was repeated in methanol but no conversion was observed in this solvent and neither was it in acetone/methanol mixtures up to 20:80 v/v. Later on, we discovered that in several other solvents (DMSO, water/methanol, THF and water/acetone 50:50 v/v) the conversion **2** → **3** did not occur. On the other hand, compound **3** did not release bromine neither in methanol nor in acetone even after heating the solution at 60 °C for several hours. Interestingly, compounds **4** and **5**, too, released bromine in *acetone* from the 8-position, the former being reconverted into quercetin. The first-order rate constant for the release of Br from the C-8 position of **2** was evaluated to be 2×10^{-6} s^{-1} at room temperature ($\tau_{1/2}$ ca. 100 h).

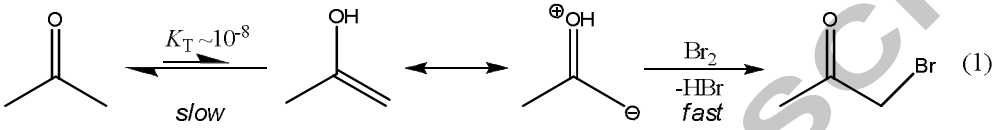
<Figure 4 about here>

Figure 5 shows that bromoquercetin **2** is able to exchange bromine with deuterium at C-8 (the new signals that appear in spectra 2 and 3, see Fig. 5, correspond to those of **3**). This was confirmed in a solvent mix acetone + acetone-d_6 50:50 v/v in which we observed the appearance of the H-8 of **3** with a peak area that initially increased (max on the 6th day) and then decreased converging toward an equilibrium composition C-H/C-D dictated by the enthalpies of these two bonds, see Figure 6. Figures 4 and 5 also show that the exchange of bromine with deuterium in compound **2** was significantly slower than the exchange with H. From these spectra, we evaluated a kinetic isotope effect (KIE) k_H/k_D of about 2, see Figs. 4 and 5.

<Figures 5 and 6 about here>

One distinctive feature of acetone is its ability to enolize, eq. 1.[17] This conversion is efficiently catalyzed by acids and bases but the amount of enol generated at equilibrium is very tiny, ≤ 0.1 ppm ($K_T = 6\times10^{-9}$ in water at 25 °C).[18] However, the double bond of the enol is electron-rich

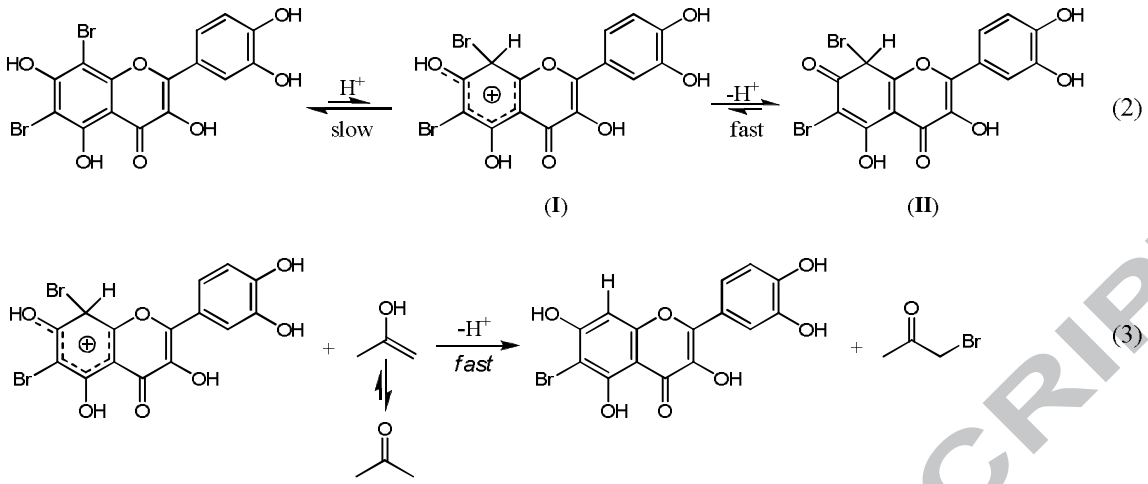
because of the carbanion character of the β-carbon, see eq. 1.[17,18] It thus reacts extremely rapidly with electrophiles such as halogens. Bromine, in particular, is a very reactive electrophile and is therefore enormously reactive toward the enol of acetone. Thus, the slow enolization of acetone is considered to be rate-determining. This is in accordance with the rate-law which, at sufficiently large [Br_2], is zeroth-order in Br_2, that is, is independent of its concentration.[19,20]



We think that the acid-catalyzed Br/H exchange observed in acetone with compounds **2**, **4** and **5** is due to bromination of the enol of acetone.[21] The involvement of this species was nicely suggested by the fact that other ketones with α-hydrogens (methyl ethyl ketone, isophorone, acetylacetone) caused the same Br/H exchange whereas fenchone (a ketone *without* α-hydrogens and thus unable to enolize) did not, see Scheme 3.[21]

<Scheme 3 about here>

Bromination of acetone by **2**, **4** and **5** differs under many aspects from the process with Br_2, eq. 1. First, the rate of enolization in our case is *not* rate-determining since when the reaction was studied in acetylacetone, whose enolic form is largely predominant,[22] we did not observe any increase in the rate. Formation of the brominating agent seems therefore to be the rate-determining step. We think that the brominating agent can be the arenium ion **I** represented in eqs. 2 and 3.



This species is certainly a powerful electrophile and can react very quickly with enols, eq. 3. That this species might be involved in the rate-determining step is also suggested by a substantial KIE of ~ 2 observed experimentally with compound **2** in acetone and acetone-d_6, see Figs. 4 and 5. Finally, the results observed with the H/D exchange, under acid catalysis, support the mechanism outlined in reactions 2 and 3. The high regioselectivity at C-8 observed in those experiments confirms that protonation occurs essentially at position 8 only, see Scheme 2.

In conclusion the C-X (X = H, D, Br) bonds at 6- and 8-positions of quercetin and quercetin derivatives are rather "loose" and thus easily replaceable through electrophilic reactions. These two positions have in fact a large π-electron density attested by their ^{13}C NMR δ values which are up-field shifted relative to the B-ring signals. Base-catalyzed H/D exchange occurs at these positions comparatively quickly in acetone ($\tau_{1/2} \approx 53$ h at room temperature) but with low regioselectivity. On the other hand, acid-catalyzed H/D exchange is slow ($\tau_{1/2}$ = 345 h in methanol and 1379 h in acetone at room temperature) but is characterized by a high regioselectivity toward the 8-position. Bromoquercetins **2**, **4** and **5** undergo a singular exchange of Br with H at 8-position when these compounds are solubilized in *enolizable* ketones. The Br/H exchange is due to bromination of the enols and the kinetics is controlled by the slow formation of the "brominating agent" which is

supposed to be the arenium ion **I** represented in eq. 2. This species is most likely a powerful electrophile which can react very quickly with enols, eq. 3. Bromoquercetins might therefore be used as convenient brominating agents since their oxidizing properties, contrary to *N*-bromosuccinimide, are expected to be poor.

Supplementary data

Supplementary data (NMR spectra of **1 – 5** in acetone-d6) are available.

References and notes

1. Foti, M. C.; Rocco, C. *Tetrahedron Lett*. **2014**, *55*, 1602 – 1607 and references therein.
2. Smith, M. B.; March, J. in *Advanced Organic Chemistry*; John Wiley & Sons: Hoboken, 2007, sixth edition.
3. Mulder, P.; Arends, I. W. C. E.; Santoro, D.; Korth, H.-G. *J. Org. Chem*. **2003**, *68*, 4247-4257.
4. Boersma, M. G.; Vervoort, J.; Szymusiak, H.; Lemanska, K.; Tyrakowska, B.; Cenas, N.; Segura-Aguilar, J.; Rietjens, I. M. C. M. *Chem. Res. Toxicol*. **2000**,*13*, 185-191.
5. Awad, H. M.; Boersma, M. G.; Boeren, S.; van Bladeren, P. J.; Vervoort, J.; Rietjens, I. M. C. M. *Chem. Res. Toxicol*. **2003**, *16*, 822-831.
6. The relaxation times T_1 of the aromatic hydrogens of the A- and B-rings were determined in acetone-d_6 and methanol-d_4 containing D_2O, H_2O, DCl or NaOD. The values ranged from 0.95 (H-5' in methanol-d_4 and in acetone-d_6 containing 13% v/v H_2O) to 5.00 seconds (H-6 in acetone-d_6 containing DCl or NaOD in D_2O). The recycle delay d1was therefore set to 20 seconds (90° pulse) long enough to ensure an accurate integration of the peaks.
7. Jones, W. D. *Acc. Chem. Res*. **2003**, *36*, 140-146.
8. The data were fitted with a *first-order* kinetic model, $\ln I_t = \ln I_0 - k_{obs} \times t$ where I is the integral of the peak, k_{obs} the observed rate constant and t the time. Given that the reaction order in DCl is 1, $k_{obs} = k \times [\mathrm{DCl}]$ where k is the second order rate constant for the H/D exchange.
9. In our experiments, a very modest exchange at 2'-position was also observed. Interestingly, compounds of gold ($HAuCl_4$) are reported to cause H/D exchange at 2'-position of quercetin, see: Shestakov, A. F.; Chernyak, A. V.; Lariontseva, N. V.; Golovanova, S. A.; Sadkov, A. P.; Levchenko, L. A. *Mendeleev Commun*. **2013**, *23*, 98–100.
10. Madhusudanan, K. P.; Sachdev, K.; Harrison, D. A.; Kulshreshtha, D. K. *Int. J. Mass Spectrom. Ion Processes* **1984**, *62*, 289-295.

11. Jordheim, M.; Fossen, T.; Songstad, J.; Andersen, Ø. M. *J. Agric. Food Chem.* **2007**, *55*, 8261–8268.

12. Faizi, S.; Siddiqi, H.; Naz, A.; Bano, S.; Lubna *Helv. Chim. Acta* **2010**, *93*, 466-481.

13. The data reported in Table 1 of ref. 11 yield a first-order rate constant, for the H/D exchange at C-8 of a quercetin 3-*O*-glycoside dissolved in CF_3COOD/MeOD (5:95 v/v), of ~1.39×10^{-6} s^{-1}.

14. Because of the presence of the base, the solutions were de-aerated with argon to avoid oxidation of the quercetin anions.

15. The mechanism involving the cyclohexadienone intermediate might also be responsible for the exchange even in neutral or weakly acidic conditions produced by deprotonation of the 7-OH, as sketched in Scheme 4 of ref. 1. In this regard, the expression "…containing DCl", reported in the caption of the scheme by mistake, must not be considered.

16. We also attempted to use 200 mM NaOH as a catalyst but we observed formation of an unknown compound and other unexpected NMR signals possibly due to degradation of compound **2**.

17. Reichardt, C. in *Solvents and Solvent Effects in Organic Chemistry*; Wiley-VCH, 2003, 3th ed.

18. Carey, F. A.; Giuliano, R. M. in *Organic Chemistry*; McGraw-Hill, New York 2011, eighth edition.

19. Bell, R. P.; Davis, G. G. *J. Chem. Soc*. **1964**, 902-904.

20. In general, the rate of acid-catalyzed halogenation of acetone (or other enolizable ketones) is independent of the nature (Cl_2, Br_2 and I_2) and concentration of the halogen when its concentration is large. At low halogen concentrations, however, the rate *does* depend on both nature and concentration. See, Bell, R. P.; Yates, K. *J. Chem. Soc*. **1962**, 1962-1933.

21. Bromination of acetone, methyl ethyl ketone, isophorone and acetylacetone was confirmed via GC-MS chromatography. The solutions were first passed on an alumina cartridge using

pentane as an eluent and then were injected to the GC-MS instrument. All ketones were used in pure form except for fenchone which required the use of a co-solvent (THF 50% v/v) because of the low solubility of **2** – **5**. The release of bromine from C-8 was followed by integrating the NMR signal of the incoming proton (H-8) relative to the B-ring. No release of bromine was observed from the C-6 position in these experiments. These studies were carried out at 60 °C in an NMR tube containing a coaxial capillary filled with D_2O for the frequency lock.

22. The equilibrium constant K_T = [enol]/[keto] at room temperature for the keto ⇆ enol equilibration of acetylacetone in acetone is 3.40 (calculated from the ^{1}H NMR spectrum in acetone). Thus, more than 77% of acetylacetone is in the enol form.

Captions to Figures and Schemes

Figure 1. 1) ^{1}H NMR spectrum of quercetin (10 mM) in methanol-d_4 containing DCl (6 mM) at room temperature. 2) After 3 days. The H-8 signal at 6.46 ppm integrated to 0.85 (relative to the H-5' doublet at 6.95); H-6 at 6.25 ppm integrated to 0.98. 3) After 22 days. The H-8 signal reduced to 0.36 while H-6 integrated to 0.93.

Figure 2. 1) ^{1}H NMR spectrum of quercetin (10 mM) in methanol-d_4 containing NaOD (6 mM) at room temperature. 2) After 22 hours. The H-8 signal at ca. 6.3 ppm integrated to 0.82 (relative to the H-5' doublet at 6.92); H-6 at 6.11 ppm integrated to 0.91. 3) After 2 days H-8 integrated to 0.36 while H-6 to 0.86. 4) After 9 days H-8 integrated to 0.32 while H-6 to 0.74.

Figure 3. 1) ^{1}H NMR spectrum of quercetin (10 mM) in acetone-d_6 containing NaOD (6 mM) at room temperature. 2) After 20 hours the signals at ca. 6.15 and 5.9 ppm (H-8 and H-6, respectively) integrated (relative to the H-5' doublet at 6.85 ppm) to 0.70 and 0.65, respectively. 3) After 2 days the signals integrated to 0.54 and 0.49, respectively.

Figure 4. 1) ^{1}H NMR spectrum of 6,8-dibromoquercetin **2** (14 mM) in non-deuterated acetone containing HCl (200 mM). The solution was placed in an NMR tube containing a coaxial capillary filled with D_2O for the frequency lock. 2) After 7 days, **2** converted into **3** for about 70%. The peaks at 7.74, 7.65, 6.94 and 6.8 ppm correspond to H-2', H-6', H-5' and H-8, respectively, of bromoquercetin **3**. 3) After 12 days a total conversion of **2** into **3** was observed (see text).

Figure 5. 1) ^{1}H NMR spectrum of 6,8-dibromoquercetin **2** (14 mM) in acetone-d_6 containing DCl (200 mM). 2) After 18 days. Partial conversion of **2** into **3** after replacement of Br with D at C-8. 3) After 25 days, traces of the initial bromoquercetin **2** were still present. The process of Br/D

exchange was significantly slower than that of Br/H exchange by a kinetic isotope effect of ~ 2, see Figure 4.

Figure 6. 1) ^{1}H NMR spectrum of 6,8-dibromoquercetin **2** (14 mM) in acetone + acetone-d_6 50:50 v/v containing DCl (200 mM). 2) After 6 days ca. 30% of **2** converted into **3** (for the peak assignment see Figure 4). The peak at 6.8 ppm corresponding to H-8 of **3** integrated (relative to the H-5' doublet at 7.0 ppm) to 0.58. 3) After 10 days the peak at 6.8 ppm integrated to 0.46 (see text).

Scheme 1. Quercetin (**1**) and bromoderivatives **2 – 5** utilized in the present study.

Scheme 2. Mechanisms of H/D exchange at 8-position of quercetin. Similar mechanisms also apply for the 6-position (under base-catalysis) and for the H/D exchange in the bromoderivatives **3** and **4**.

Scheme 3. Ketones used as solvents in the experiments of bromine release with **2** – **5**.

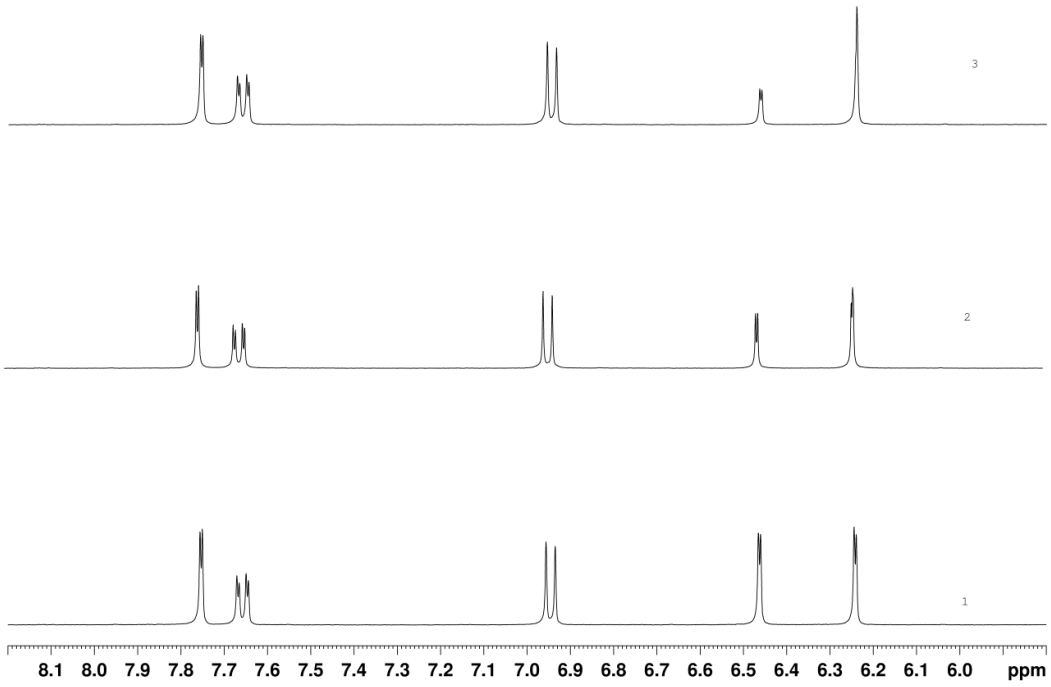


Figure 1

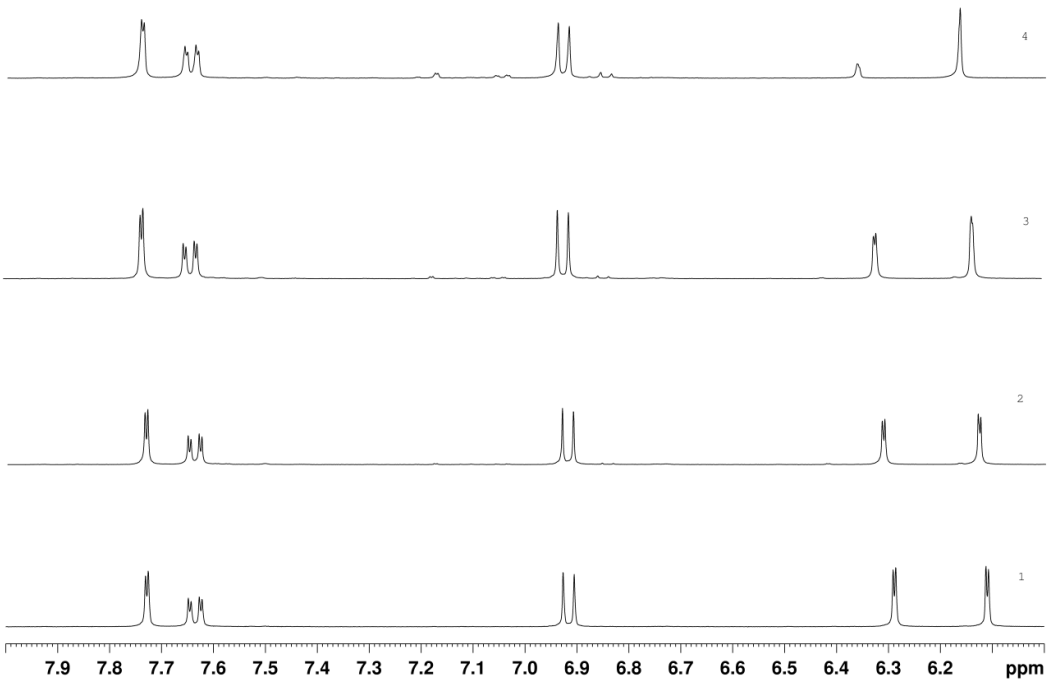


Figure 2

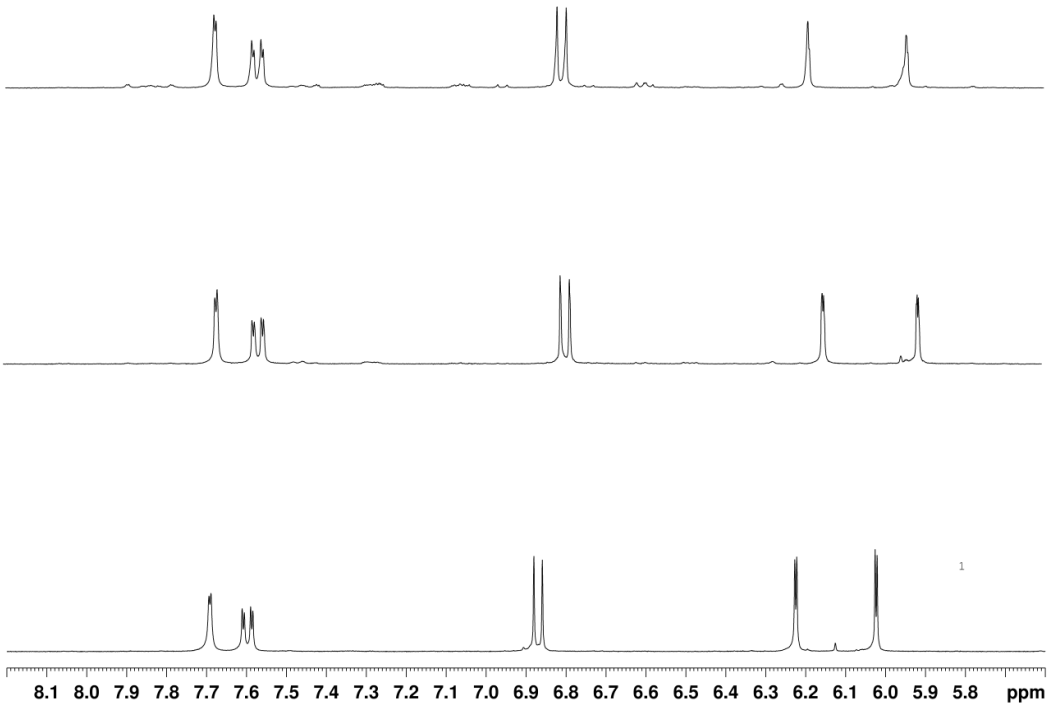


Figure 3

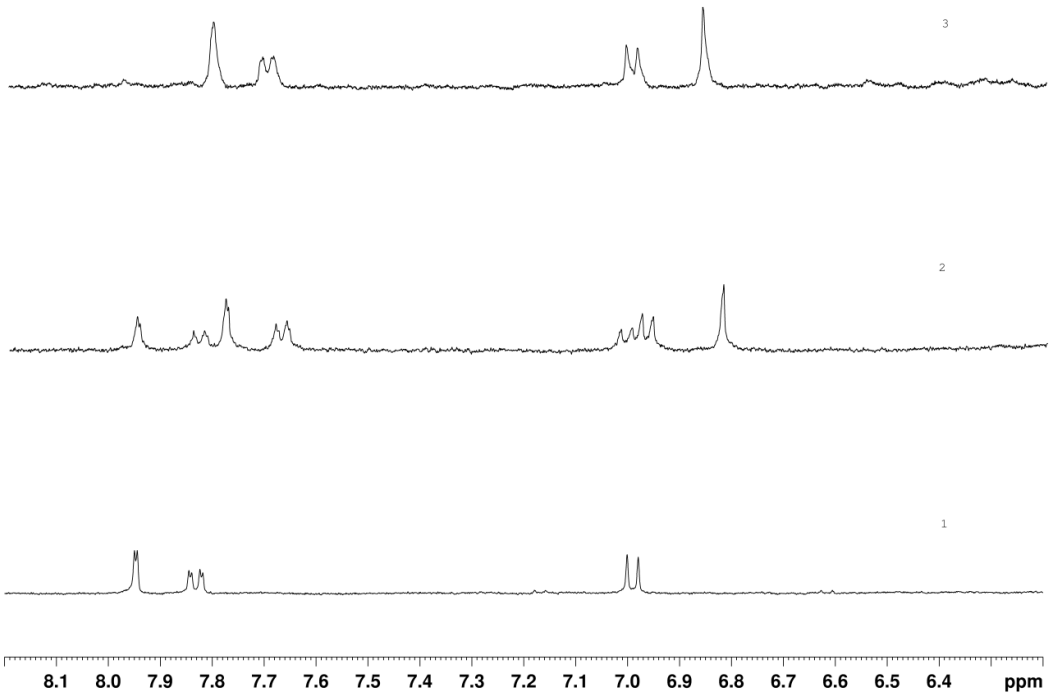


Figure 4

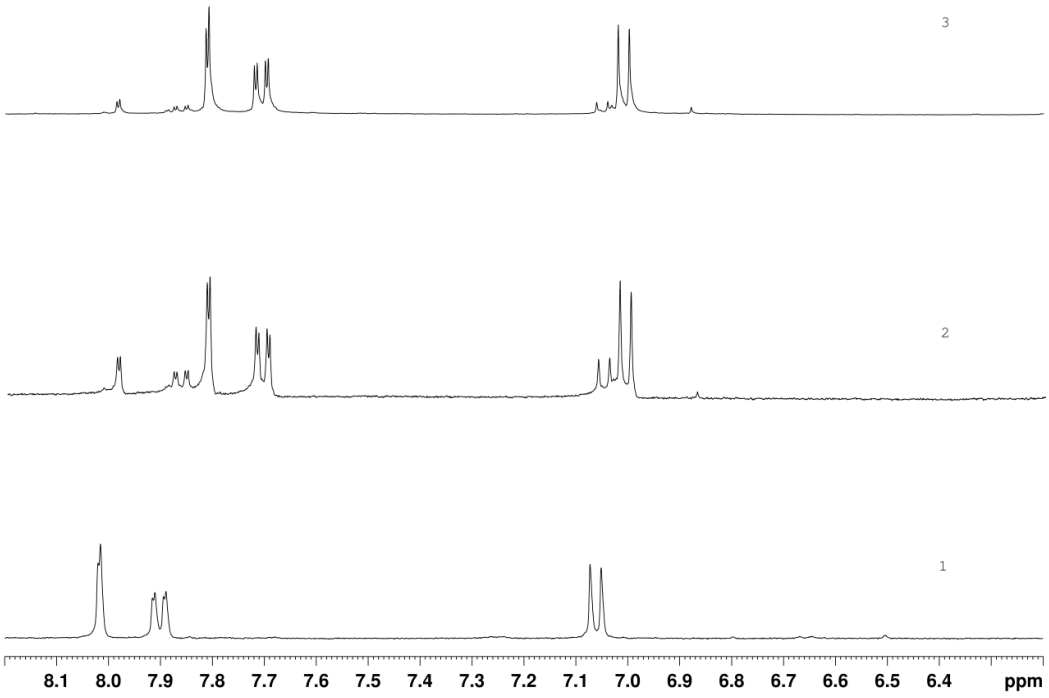


Figure 5

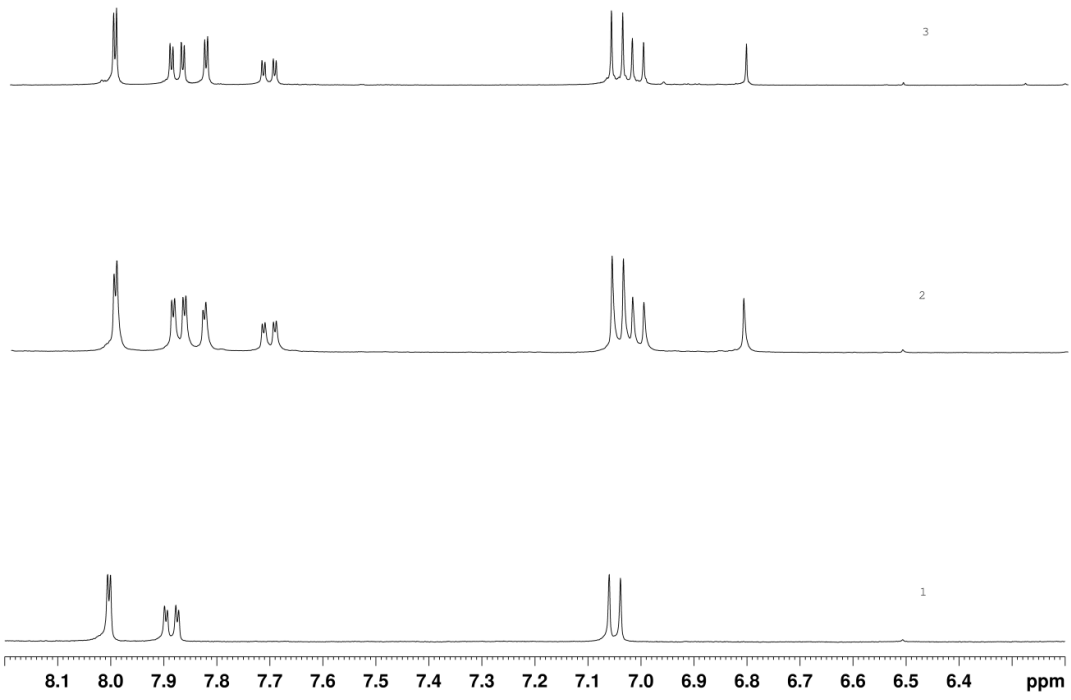
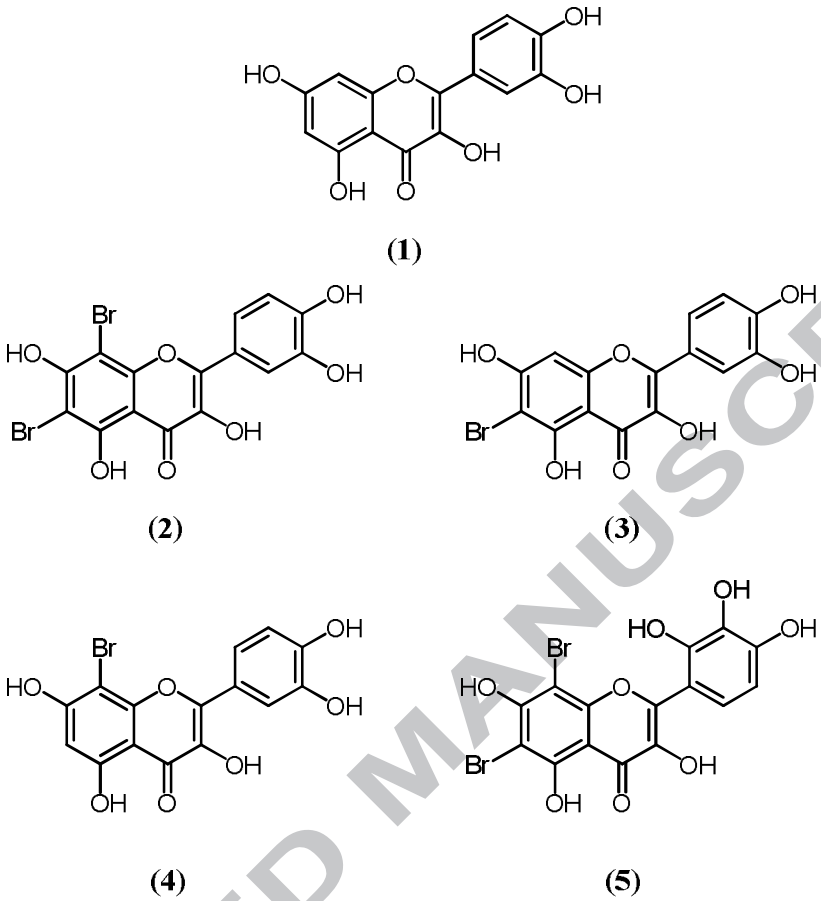
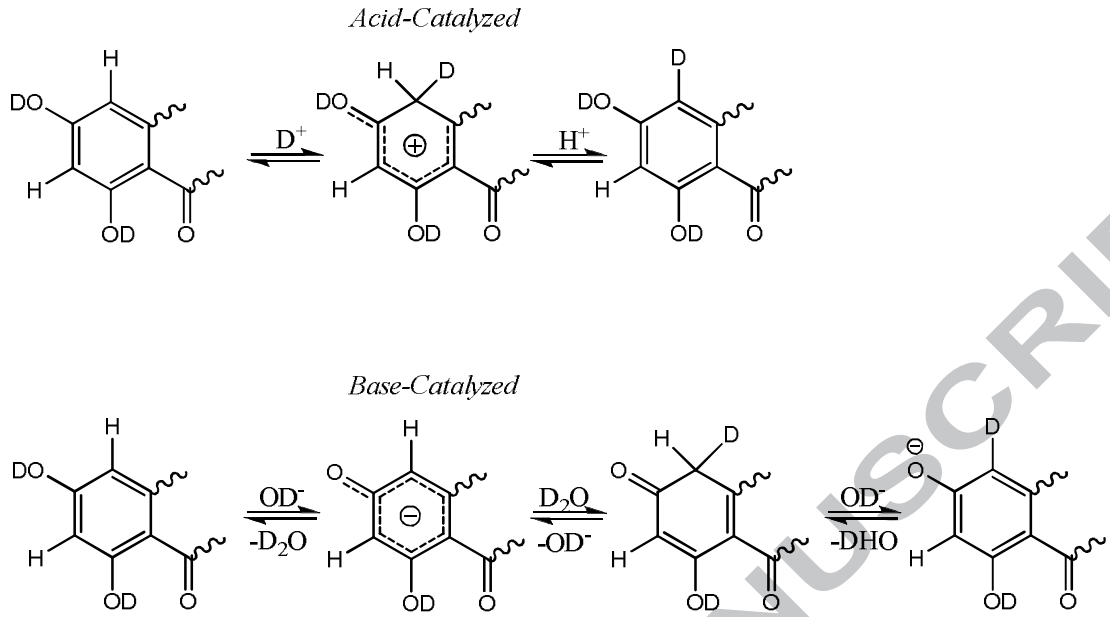


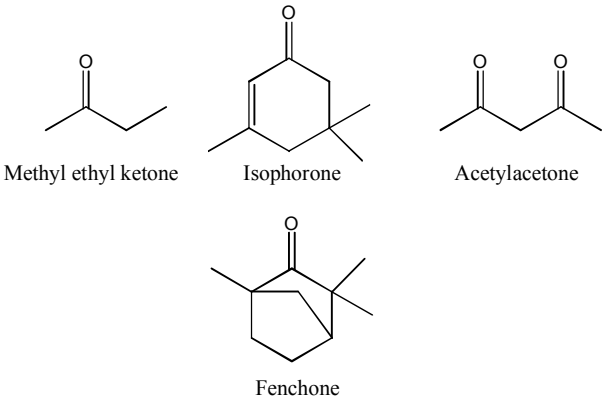
Figure 6



Scheme 1



Scheme 2



Scheme 3

Table 1. Observed rate constants (s^{-1}) and $\tau_{1/2}$ (h) (in parentheses) for H/D exchange of quercetin H-8 and H-6 at room temperature.

	k_{obs}/s^{-1} ($\tau_{1/2}/h$)[a] 6 mM DCl		**k_{obs}/s^{-1} ($\tau_{1/2}/h$)[a] 6 mM NaOD**	
	Acetone-d_6	**Methanol-d_4**	**Acetone-d_6**	**Methanol-d_4**
H-8	1.39×10^{-7} (1379)	5.56×10^{-7} (345)	3.39×10^{-6} (56.5)	1.67×10^{-6} (115)
H-6	*very small*	*very small*	3.94×10^{-6} (48.6)	2.78×10^{-7} (689)

[a]Error estimated to be ca. ±10%.

Graphical abstract