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# Stoichiometric oxidation of quercetin by $HAuCl_4$ accompanied by H–D exchange with the solvent

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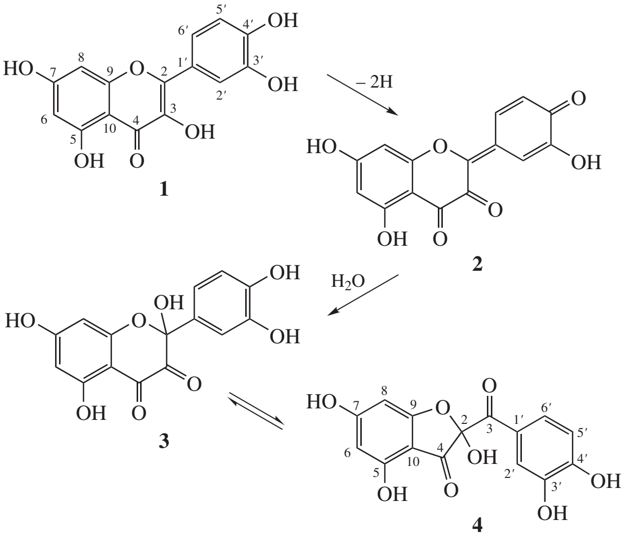
**The stoichiometric two-electron oxidation of quercetin by $HAuCl_4$ is accompanied by H–D exchange with $D_2O$ at the 2′-position, which was not observed previously.**

Recently,[1] we found that the interaction of $HAuCl_4$ with bioflavonoids in aqueous solutions led to their deep oxidation. To study the reaction between quercetin $C_{15}O_7H_{10}$ and $HAuCl_4$ at room temperature (22±2 °C), we used NMR spectroscopy.[†] For this purpose, a 0.1 M solution of quercetin was prepared in a mixture of DMSO-$d_6$ and $D_2O$ (4 : 1, by volume); then, $HAuCl_4$ was added to this solution in two steps at regular intervals of four days to obtain quercetin-to-Au ratios of 1 : 0.5 and 1 : 1 after the first and second steps, respectively.

To obtain the $^1$H NMR spectra, a standard pulse sequence $\pi/2$ free induction decay (FID) was used. For the accumulation of $^{13}$C NMR spectra, a standard pulse sequence $\tau$ $\pi/6$-SSI was used with the suppression of $^1$H during the entire experiment. The delay before the pulse $\tau$ was 1 s, and the number of savings was 500–4000. Standard two-dimensional correlations $^1$H-$^1$H COSY, $^1$H-$^1$H NOESY, $^{13}$C-$^1$H HSQC, $^{13}$C-$^1$H HMBC were studied to interpret the spectra. Lines due to DMSO at 2.51 ppm ($^1$H) and 39.51 ppm ($^{13}$C) were used as reference ones. Earlier, in the investigation of quercetin **1** oxidation by air,[2] it was found that the presence of $Fe^{III}$ or $Cu^{II}$ ions in the system accelerates the reaction. *p*-Quinonemethide **2** is formed, which quickly adds water molecule with the generation of a cyclic semiketal, which occurs in two forms **3** and **4** (Scheme 1).



**Scheme 1**

Slow oxidation of quercetin under the action of $Cu^{II}$ ions was reported,[3] but the reaction products were not identified.

The $^1$H and $^{13}$C NMR spectra of quercetin (Table 1) are consistent with published data.[4–6] Signals of O and Cl nuclei were not revealed because of their significant broadening. When adding $HAuCl_4$, a drift of $H^6$ and $H^8$ quercetin peaks in the $^1$H NMR spectra was no more than 0.02 ppm. Moreover, this shift increases with time under increasing degree of replacement of H by D in these positions. Thus, this effect is probably due to the influence of deuterium, and, most likely gold complexes with quercetin do not form under experimental conditions. Such complex formation, for example, in the case of $Cu^{II}$, leads to the observed shift of proton signals up to 0.5 ppm towards low fields.[5]

We found 15 additional lines in the $^{13}$C NMR spectrum after adding the first portion of $HAuCl_4$ (Figure 1). This indicates the presence of only two organic compounds in the system: quercetin and its oxidation product. Thus, the equilibrium in Scheme 1 is completely shifted to one side. According to a detailed study of the electrochemical oxidation of quercetin,[7] it acts as a two-electron reducer in an acid medium. Since $HAuCl_4$ is a complementary two-electron oxidizing agent, the primary redox reaction (1)

$$C_{15}O_7H_{10} + Au^{3+} = C_{15}O_7H_8 + Au^+ + 2H^+ \qquad (1)$$

proceeds rather quickly, and the degree of conversion $\eta$ = 46% is achieved within 10 min (Table 2). The degree of transformation was monitored by a change of the signal intensities of the original compound and its oxidation product in the $^1$H NMR spectra. This gives a rough estimate of the effective rate constant of reaction (1) at ~$10^{-2}$ mol dm$^{-3}$ s$^{-1}$. After the second addition of $HAuCl_4$, only signals from the oxidized form remain in the $^{13}$C NMR spectrum. Thus, the oxidation of quercetin by $HAuCl_4$ is stoichiometric with the formation of a single product. It is accompanied by a change in the optical spectrum of the system and can be used to develop a sensitive analytical procedure for the determination of Au.[8]

The reliable assignment of signals was carried out taking into account the two-dimensional $^{13}$C–$^1$H correlations HSQC and HMBC, which indicate that only carbon atom at 105.2 ppm does not have any interaction, even distant, with hydrogen atoms. That is, carbon at 105.2 ppm is distant at least by four bonds from any hydrogen atom. Only form **4** of the two equilibrium forms of the oxidized quercetin meets this requirement.

On the two-electron oxidation of quercetin, the formation of two structures is possible, *o*-quinone one (the oxidation of two *ortho* OH groups in ring B), and *p*-quinone one (shown in Scheme 1). According to quantum-chemical calculations performed using

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[†] The $^1$H and $^{13}$C NMR spectra were recorded on a Bruker Avance III Fourier NMR spectrometer (500 and 126 MHz, respectively).

**Table 1** Experimental and theoretical shielding constants of $^1H$ and $^{13}C$ nuclei for quercetin and its oxidation product.

| Atom C(H) | Quercetin **1** (experiment) | | Oxidation product **4** | | | |
|---|---|---|---|---|---|---|
| | | | $\delta_{^1H}$/ppm | | $\delta_{^{13}C}$/ppm | |
| | $\delta_{^1H}$/ppm | $\delta_{^{13}C}$/ppm | Experiment | Theory | Experiment | Theory |
| 2 | — | 146.9 | — | | 105.2 | 125.4 |
| 3 | — | 135.8 | — | | 191.1 | 196.5 |
| 4 | — | 175.9 | — | | 191.0 | 197.4 |
| 5 | — | 160.8 | — | | 159.2 | 168.2 |
| 6 | 6.16 ($^4J_{H^6-H^8}$ 2.1 Hz) | 98.3 | 5.94 ($^4J_{H^6-H^8}$ 1.8 Hz) | 5.63 | 97.4 | 97.1 |
| 7 | — | 164.0 | — | | 169.3 | 174.7 |
| 8 | 6.41 ($^4J_{H^6-H^8}$ 2.1 Hz) | 93.5 | 5.94 ($^4J_{H^6-H^8}$ 1.8 Hz) | 5.26 | 91.3 | 90.5 |
| 9 | — | 156.2 | — | | 172.7 | 178.0 |
| 10 | — | 103.1 | — | | 101.2 | 105.7 |
| 1′ | — | 122.1 | — | | 125.7 | 132.5 |
| 2′ | 7.60 ($^4J_{H^{2'}-H^{6'}}$ 2.3 Hz) | 115.2 | 7.48 ($^4J_{H^{2'}-H^{6'}}$ 2.2 Hz) | 8.56 | 117.9 | 124.2 |
| 3′ | — | 145.1 | — | | 145.4 | 151.9 |
| 4′ | — | 147.7 | — | | 152.0 | 154.4 |
| 5′ | 6.88 ($^3J_{H^{5'}-H^{6'}}$ 8.5 Hz) | 115.7 | 6.81 ($^3J_{H^{5'}-H^{6'}}$ 8.2 Hz) | 6.35 | 115.7 | 114.1 |
| 6′ | 7.51 ($^3J_{H^{5'}-H^{6'}}$ 8.5 Hz, $^4J_{H^{2'}-H^{6'}}$ 2.3 Hz) | 120.1 | 7.49 ($^3J_{H^{5'}-H^{6'}}$ 8.2 Hz, $^4J_{H^{2'}-H^{6'}}$ 2.2 Hz) | 7.38 | 124.8 | 125.7 |

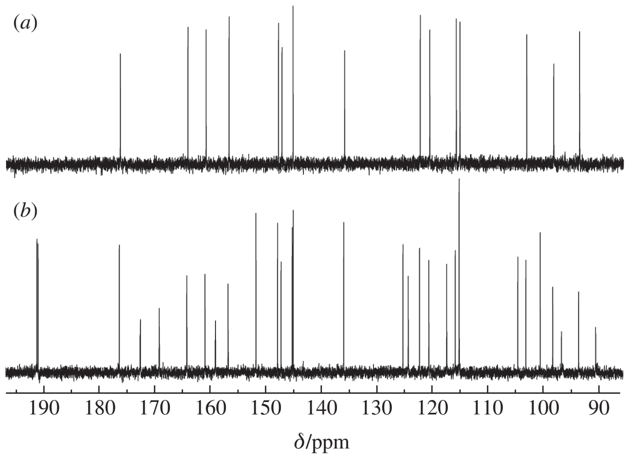

**Figure 1** $^{13}C$ NMR spectra of quercetin (*a*) before adding $HAuCl_4$ and (*b*) after the first adding of $HAuCl_4$.

density functional theory with nonempirical PBE functional with extended basis: H [6*s*2*p*/2*s*1*p*] C, O [10*s*7*p*3*d*/3*s*2*p*1*d*],[‡] the energy of the latter structure is lower than that of the *o*-quinone one by 7.9 kcal mol$^{-1}$. The binding of a water molecule with the formation of structure **4** results in an energy release of 8.1 kcal mol$^{-1}$. The energy of isomeric structure **3** is higher by 1.7 kcal mol$^{-1}$ ($\Delta G$ = 1.9 kcal mol$^{-1}$). The calculated chemical shifts for $^1H$ and adjacent $^{13}C$ nuclei in structure **4** (Figure 2) are in reasonable agreement with experimental values (Table 1). The assignments of other signals in the $^{13}C$ NMR spectrum of **4**, except for the $C^2$ atom, was performed taking into account the theoretical chemical shifts. The mean deviation theory–experiment is similar to the case of C atoms with C–H bonds.

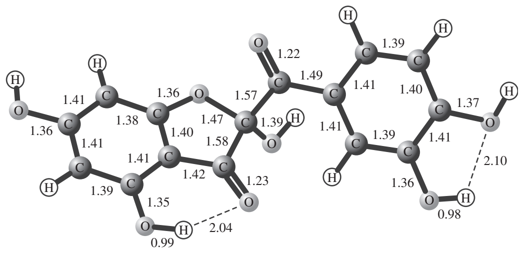

**Figure 2** Structure of the product **4** of two-electron oxidation of quercetin according to the quantum-chemical calculations. Bond lengths are given in Å.

In quercetin and its oxidation product **4** H–D exchange with the solvent occurs mainly in the 8- and 6-positions (Table 2). Earlier such exchange was observed in quercetin in $CF_3COOD$ solutions,[10] but the 2′-position was not affected. $HAuCl_4$ is responsible for these phenomena because in its absence H–D exchange in quercetin was not detected in six days. The estimated effective exchange rate constants of the first order are 7×10$^{-6}$, 4×10$^{-6}$ and 3×10$^{-6}$ s$^{-1}$ for the 8-, 6- and 2′-positions, respectively. They are much smaller than those, more or about 10$^{-3}$ s$^{-1}$, found for exchange in $CF_3COOD$.[8] This difference is justified, since the concentration of protons in the system, taking into account the formation of HCl, is about 0.15 mol dm$^{-3}$, which is one order of magnitude lower than that in $CF_3COOD$. The mechanism of H–D exchange at the 6- and 8-positions seems to comply with keto-enol tautomerism, as suggested previously.[10]

Our calculations show that the energy of the isomeric form of quercetin with a carbonyl group at the 7-position and an additional H atom in the 8- or 6-position is higher by 11.2 or 14.4 kcal mol$^{-1}$, respectively, than the energy of quercetin. The similar isomerization of oxidized product **4** requires 12.0 and 14.3 kcal mol$^{-1}$, respectively. This is in qualitative agreement with a faster exchange at the 8-position of quercetin. However, the degrees of exchange for

**Table 2** Kinetics of exchange of quercetin protons with deuterium.

| Time/h | $\eta^a$ (%) | Deuterium exchange of protons in **1** (%) | | | | | Deuterium exchange of protons in **4** (%) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 8 | 6 | 2′ | 6′ | 5′ | 8 | 6 | 2′ | 6′ | 5′ |
| 0.17 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 5 | 83 | 12 | 8 | 5 | 0 | 0 | 17 | 17 | 7 | 0 | 0 |
| 26 | 100 | 18 | 7 | 5 | 0 | 0 | 30 | 30 | 6 | 0 | 0 |
| 32 | 100 | 22 | 7 | 7 | 0 | 0 | 35 | 35 | 5 | 0 | 0 |
| 48 | 100 | 27 | 9 | 9 | 0 | 0 | 49 | 49 | 9 | 0 | 0 |
| 73 | 100 | 37 | 11 | 8 | 0 | 0 | 64 | 64 | 12 | 0 | 0 |
| 144 | 100 | 62 | 16 | 12 | 0 | 0 | 88 | 88 | 13 | 0 | 0 |
| 168 | 100 | 68 | 18 | 13 | 0 | 0 | 91 | 91 | 14 | 0 | 0 |
| 670 | 100 | 94 | 29 | 14 | 0 | 0 | 96 | 96 | 19 | 0 | 0 |

$^a\eta$ is the conversion of quercetin in the reaction with $HAuCl_4$ (the reaction was completed at the ratio **1** : **4** = 1 : 1).

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[‡] Quantum-chemical calculations have been made using the PRIRODA program[9] at the facilities of the Joint Supercomputer Center of the Russian Academy of Sciences.

the 6- and 8-positions in the product are the same, which indicates fast intramolecular exchange between the 6- and 8-positions in **4**.

The exchange in the 2′-position takes place only in the presence of Au compounds. One can assume that the activation of a C–H bond in ring B is responsible for it based on the fact that even aliphatic C–H bonds are activated in the Au–bioflavonoid (rutin, quercetin) systems.[11,12]

The oxidized form of quercetin in the absence of gold is characterized by exactly the same shielding constants in $^1$H NMR spectra; thus, it, like quercetin itself does not form Au-containing complexes in appreciable quantities. Apparently, this is due to the acidic medium and the presence of highly coordinating DMSO molecules. Thus, we can conclude that in the stoichiometric reaction found the solvent complex of gold(I), like AuCl(DMSO), is produced.

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