



## Study the Active Site of Flavonoid Applying Radiation Chemistry

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### I. Introduction

Flavonoid are a large and important class of naturally occurring, low molecular weight benzo- $\gamma$ -pyrone derivatives which are reported to have a myriad of biological activities, but the study on the active sites of flavonoids is still ambiguous [1,2].

In this paper, rutin, quercetin and baicalin have been selected as model compounds. It is well known that rutin is used in inhibiting arteriosclerosis and baicalin is antibacterial and antiviral. They have similar basic structure, but their medicinal properties are so different, why? As most flavonoids contain carbonyl group, which can capture electron effectively, we predict that flavonoids can capture electron to form radical anion. The formation of anion radical may have influence on the mitochondrial electron transport chain [3]. The difference in the ability of forming anion radical may cause the difference in their medicinal effects.

### II. Experiment [1,4]

### III. Results and discussion

#### Part I. Pulse radiolysis of $N_2$ saturated rutin and baicalin ethanol solution

We use ethanol as solvent, because aglycones (such as quercetin) are slightly soluble in water. Besides, ethanol solution is rather simpler than aqueous solution.

The transient absorption spectrum at  $5\mu s$  after pulse radiolysis of  $N_2$  saturated ethanol solutions containing  $1.0 \times 10^{-4} \text{ mol dm}^{-3}$  rutin, quercetin and baicalin, respectively, are shown in Fig 1, 2, 3. The kinetic constants obtained from these experiments are as follows:

The rate constants of the reaction of rutin, quercetin and baicalin with solvated electron are  $7.5 \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ ,  $7.7 \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$  and  $1.3 \times 10^{10} \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ , respectively [1,4]. The decay rate constants of rutin anion radical, quercetin anion radical and baicalin anion radical are:

$$k_a: 1.1(\pm 0.1) \times 10^4 \text{ s}^{-1}, 1.5(\pm 0.1) \times 10^4 \text{ s}^{-1} \text{ and } 2.6(\pm 0.4) \times 10^3 \text{ s}^{-1}$$

$$k_b: 2.7(\pm 0.2) \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}, 4.0(\pm 0.5) \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1} \text{ and } 1.3(\pm 0.2) \times 10^9 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$$

It shows that the reaction rate constant with  $e_s$  of baicalin is two times greater than that of rutin and quercetin, but the decay constant of baicalin anion radical are smaller than that of rutin and quercetin anions. It reveals that baicalin anion is rather stable. Which one is easier to transport (or loss) electron? So, the effect of  $CH_3CHO$  on the decay of these radical anions has been studied. From the Fig 4, 5, 6, it is clearly shown that rutin anion can transfer electron easily, while baicalin anion not. If anion radical is more stable, probably it can break the respiratory chain irreversibly.

#### Part II. Study on the reactions of typical flavonoids with peroxy radical

Some flavonoids are used as anti-aging medicine. It is said that flavonoid can capture oxidizing species such as  $O_2^{\cdot -}$ . What is the mechanism of capturing? What are the intermediate and final products after flavonoids capturing oxidizing species, which are still lacking in studying.

##### 1. Study on the kinetics of the reactions of rutin with $HO_2^{\cdot}$ .

The rate constants of rutin and baicalin with  $\text{HO}_2\cdot$  are calculated from Fig 7, 8 as  $3.6 \times 10^7 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$  and  $7.2 \times 10^7 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1}$ .

The mechanism of scavenging  $\text{HO}_2\cdot$  suggested by Javanovic [5] do not agree with the measured rates of rutin and baicalin with  $\text{HO}_2\cdot$ , in which baicalin > rutin, but there is no hydroxyl group in the B ring of baicalin.

Besides, the  $G(\text{H}_2\text{O}_2)_{\text{max}}$  in rutin aqueous solution calculated by Javanovic's mechanism is 6.75, but actually determined in our laboratory is 10. Higher  $G(\text{H}_2\text{O}_2)$  value probably means that after capturing  $\text{RO}_2\cdot$  or  $\text{HO}_2\cdot$  flavonoid peroxide may be formed. The decomposition of organic peroxide initiates a new short chain reaction.

2. The radiation chemistry of aerated baicalin-ethanol solution.

A rather stable intermediate of baicalin has been found, its decay constant is equal to  $1.2 \times 10^{-3} \text{ min}^{-1}$  [6]. It can react with KI with G value 0.84 (approximately 1/2 G(-baicalin)) [4], which means that the intermediate is probably ROOH.

3. The identification of intermediate and final products of  $\gamma$  irradiated baicalin-ethanol solution by HPLC-MS [4].

The results show that there existed several organic peroxides and splitting of B ring took place (fig 9, 10). In the case of  $\gamma$  irradiated rutin-ethanol solution, similar results were also obtained. The decomposition of organic peroxide can initiate chain reaction, such as:



It causes cytotoxic effect by flavonoid on cell.

4. Isolation of final radiolytic products has been performed. These compounds have phenolic and quinoid structures (Fig 10) [7].

#### IV. General Conclusions

- (1) The antioxidant mechanism of flavonoids should be attributed to the peroxy radical adding to flavonoids.
- (2) The cytotoxic effect of flavonoids is possibly related to :
  1. The electron scavenging ability of flavonoids and the stability of the resulted anion radical.
  2. While peroxy radical adding to flavonoids, organic peroxide will be provided and decompose to free radical subsequently, which initiates chain propagation and causes the cytotoxicity.
  3. The final products have quinoid structure and phenolic structure, their medicinal effect should be considered.
- (3) It should be cautious for patients to consider flavonoids as health care medicine.

#### References

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**Figures**

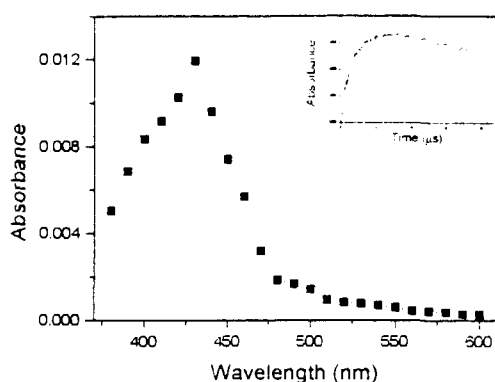


Fig.1 Transient absorption spectrum at 5 $\mu$ s after pulse radiolysis of N<sub>2</sub> saturated ethanol solution containing 1.0 $\times 10^{-4}$  mol dm<sup>-3</sup> rutin  
Inset: formation and decay of transient optical absorption at 430nm

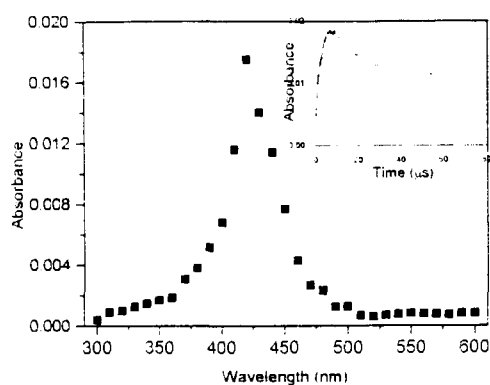


Fig.2 Transient absorption spectrum at 5 $\mu$ s after pulse radiolysis of N<sub>2</sub> saturated ethanol solution containing 1.0 $\times 10^{-4}$  mol dm<sup>-3</sup> quercetin  
Inset: formation and decay of transient optical absorption at 420nm

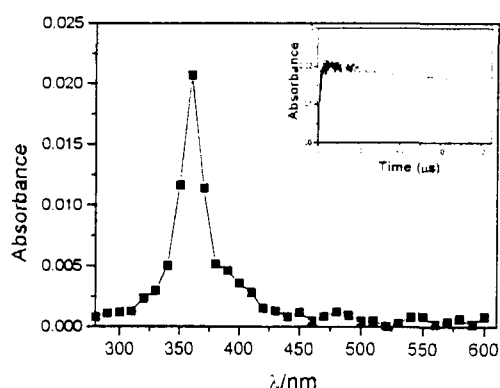


Fig.3 Transient absorption spectrum at 5 $\mu$ s after pulse radiolysis of N<sub>2</sub> saturated ethanol solution containing 1.0 $\times 10^{-4}$  mol dm<sup>-3</sup> baicalin  
Inset: formation and decay of transient optical absorption at 360nm

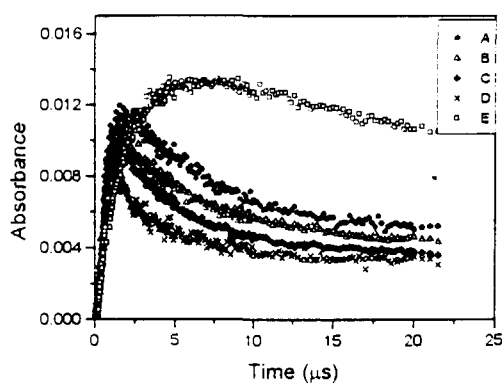


Fig.4 The buildup and decay of transient species at 430nm

Pulse dose: 7.3Gy

- A: 1.0 $\times 10^{-3}$  mol dm<sup>-3</sup> rutin and 3.56 $\times 10^{-3}$  mol dm<sup>-3</sup> CH<sub>3</sub>CHO  
 B: 1.0 $\times 10^{-3}$  mol dm<sup>-3</sup> rutin and 5.34 $\times 10^{-3}$  mol dm<sup>-3</sup> CH<sub>3</sub>CHO  
 C: 1.0 $\times 10^{-3}$  mol dm<sup>-3</sup> rutin and 7.12 $\times 10^{-3}$  mol dm<sup>-3</sup> CH<sub>3</sub>CHO  
 D: 1.0 $\times 10^{-3}$  mol dm<sup>-3</sup> rutin and 8.90 $\times 10^{-3}$  mol dm<sup>-3</sup> CH<sub>3</sub>CHO  
 E: 1.0 $\times 10^{-4}$  mol dm<sup>-3</sup> rutin

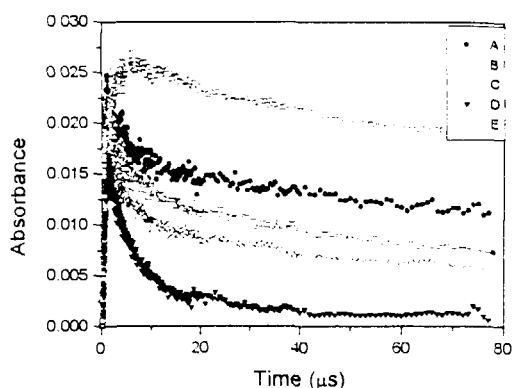


Fig.5 The buildup and decay of transient species at 420nm  
 Pulse dose: 12.3Gy  
 A:  $1.0 \times 10^{-3} \text{ mol dm}^{-3}$  quercetin and  $3.56 \times 10^{-4} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$   
 B:  $1.0 \times 10^{-3} \text{ mol dm}^{-3}$  quercetin and  $5.34 \times 10^{-4} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$   
 C:  $1.0 \times 10^{-3} \text{ mol dm}^{-3}$  quercetin and  $7.12 \times 10^{-4} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$   
 D:  $1.0 \times 10^{-3} \text{ mol dm}^{-3}$  quercetin and  $1.07 \times 10^{-3} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$   
 E:  $1.0 \times 10^{-4} \text{ mol dm}^{-3}$  quercetin

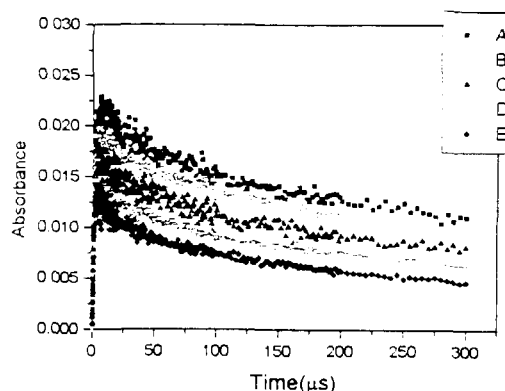


Fig.6 The buildup and decay of transient species at 360nm  
 Pulse dose: 4.8Gy  
 A:  $1.0 \times 10^{-4} \text{ mol dm}^{-3}$  baicalin  
 B:  $1.0 \times 10^{-4} \text{ mol dm}^{-3}$  baicalin and  $5.34 \times 10^{-5} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$   
 C:  $1.0 \times 10^{-4} \text{ mol dm}^{-3}$  baicalin and  $7.12 \times 10^{-5} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$   
 D:  $1.0 \times 10^{-4} \text{ mol dm}^{-3}$  baicalin and  $8.90 \times 10^{-5} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$   
 E:  $1.0 \times 10^{-4} \text{ mol dm}^{-3}$  baicalin and  $1.07 \times 10^{-4} \text{ mol dm}^{-3}$   $\text{CH}_3\text{CHO}$

The kinetic constants and thermodynamic constants of rutin and quercetin radical anions electron transfer reaction system

Substrate	$k_1 (10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1})$	$k_2 (10^8 \text{ mol}^{-1} \text{ dm}^3 \text{ s}^{-1})$	Equilibrium constant K	$\Delta E^\circ$ (V)	$\phi^\circ$ (V) of anion radical
Rutin	$2.9 \pm 0.4$	$1.3 \pm 0.2$	2.2	0.02	-1.95
Quercetin	$4.9 \pm 0.4$	$0.96 \pm 0.04$	5.1	0.04	-1.97

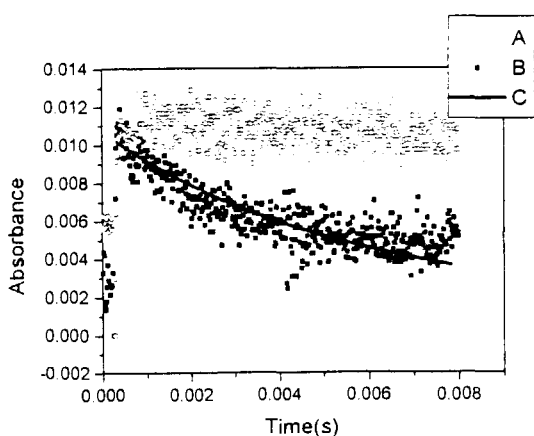


Fig.7 The decay of  $\text{O}_2^{\cdot-}$  in  $\text{O}_2$  saturated aqueous solution containing  $1.0 \times 10^{-2} \text{ mol dm}^{-3}$   $\text{HCOONa}$  and  $8.0 \times 10^{-6} \text{ mol dm}^{-3}$  rutin  
 Dose: 4.1Gy

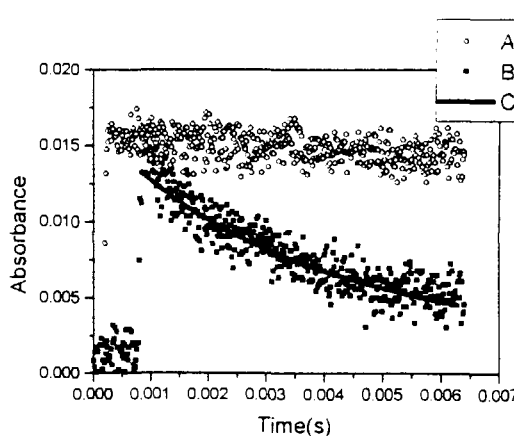


Fig.8 The decay of  $\text{O}_2^{\cdot-}$  in  $\text{O}_2$  saturated aqueous solution containing  $1.0 \times 10^{-2} \text{ mol dm}^{-3}$   $\text{HCOONa}$  and  $8.0 \times 10^{-6} \text{ mol dm}^{-3}$  baicalin  
 Dose: 5.1Gy

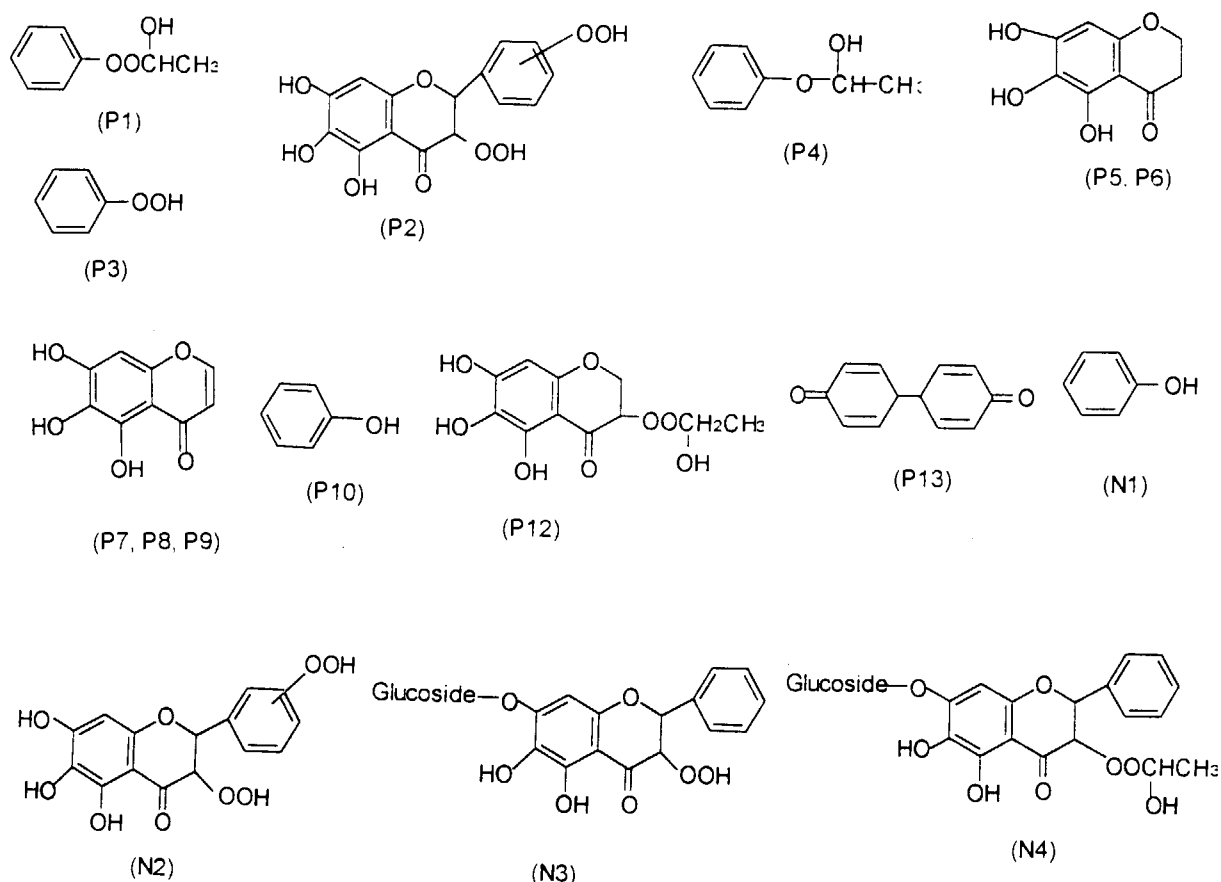
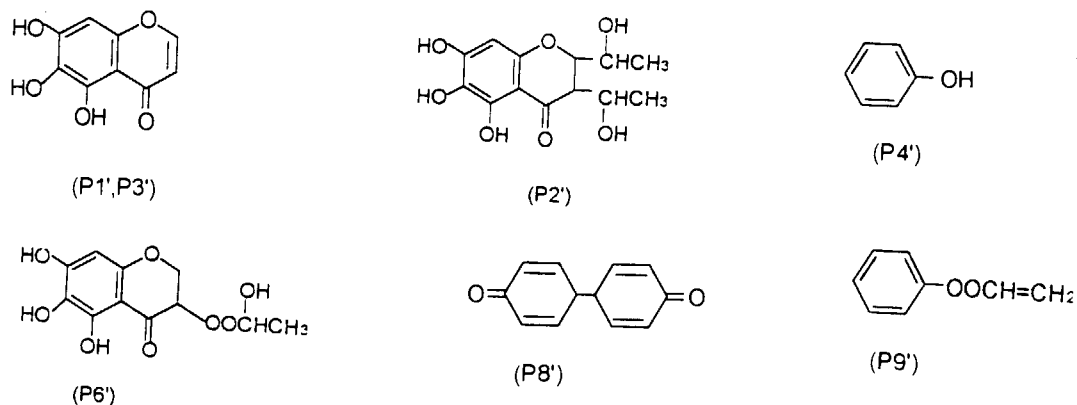


Fig.9 The identification of intermediate products

(1) The identification of final products



(2) The identification of final products by Deng

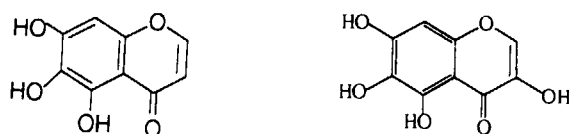


Fig.10 The identification of final products