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Structure-antioxidant capacity relationship of dihydrochalcone compounds in *Malus*

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ABSTRACT

The antioxidant capacity (AC) of six dihydrochalcone compounds was evaluated using DPPH and ABTS assays. In water-based solution 3-hydroxyphlorizin exhibited the highest AC among all dihydrochalcones. In acetone and acidic solutions (pH = 2.5 or 2.0), presence of an *o*-dihydroxyl at the B-ring increased AC, whereas glycosylation at the A-ring decreased AC of dihydrochalcones. By comparing the AC of dihydrochalcones with similar structures, it was found that the *o*-dihydroxyl at the B-ring and 2′-hydroxyl group at the A-ring were critical for maintaining the AC of dihydrochalcones by promoting hydrogen atom transfer or single electron transfer mechanism. Sequential proton-loss electron transfer commonly occurred during free radical scavenging in water-based solution. Moreover, we report a unique phenomenon in which glycosylation at the 2′-position enhanced the dissociation ability of the 4′-hydroxyl group and increased the AC of dihydrochalcones containing *o*-dihydroxyl. We speculate that this increase in AC might occur through intramolecular electron transfer.

1. Introduction

Free radicals are very reactive molecules that cause damage to the human body, as well as many chronic health problems (Forman, Davies, & Ursini, 2014; Pisoschi & Pop, 2015). Free radical scavenging by antioxidants is an important line of defense against free radical damage (Niki, 2014; Shahidi & Zhong, 2015). Flavonoids are widely found in fruits and vegetables and are considered excellent antioxidants (Bordenave, Hamaker, & Ferruzzi, 2014; Gomes de Moura & Ribeiro, 2017). The antioxidant capacity (AC) is used to evaluate the antioxidant potency of flavonoids and is defined as the amount of free radicals scavenged by antioxidant compounds (Ghiselli, Serafini, Natella, & Scaccini, 2000; Prior, Wu, & Schaich, 2005). Therefore the AC reflects the chemical equilibrium of reaction between antioxidants and free radicals. The AC of flavonoids is dependent upon the presence of hydroxyl groups at specific positions on the flavonoid skeleton (Amic et al., 2014; Mazzone, Galano, Alvarez-Idaboy, & Russo, 2016). Various action mechanisms are involved in the process of quenching free radicals by flavonoids (Galano et al., 2016), and three of them are considered as the primary antioxidant actions (Amic et al., 2014, 2017; Mazzone, Malaj, Galano, Russo & Toscano, 2015). One of these is hydrogen atom transfer (HAT), which is a one-step reaction governed by the O−H bond dissociation enthalpy (BDE). The other two pathways are single electron transfer followed by proton transfer (SET-PT) and sequential proton-loss electron transfer (SPLET). SET-PT and SPLET are two-step reaction processes, with the first step being governed by the ionization potential (IP) and proton affinity (PA), respectively (Scheme 1; Stepanić, Trošelj, Lučić, Marković & Amić, 2013; Vagánek, Rimarčík, Dropková, Lengyel & Klein, 2014). Moreover, HAT is preferred in non-polar solvents. For SET-PT pathway it is more convenient to occur in polar aqueous than non-polar solvents (Stepanić et al., 2013), while SPLET occurs in ionizing solvents (Litwinienko & Ingold, 2003; Amić et al., 2017). These free radical scavenging mechanisms can occur simultaneously, with the radical adduct formation being also possible (Shadnia & Wright, 2008). The total AC may include various mechanisms (Klein, Rimarčík, Senajová, Vagánek & Lengyel, 2016). Reaction conditions, free radical type, and flavonoid chemical structure determine which mechanisms predominant.

Dihydrochalcones (DHCs) are an important subgroup of flavonoids in apple fruit (Tsao, Yang, Young & Zhu, 2003; Lin, Hsu, Chen, Chern & Lee, 2007; Chen, Zhang, Wang, Li & Ma, 2012) and have good antioxidant potencies (Xiao et al., 2017). DHCs have a basic C_6-C_3-C_6 skeleton chemical structure. The A-ring and B-ring are not conjugated together like most flavonoids, but are instead linked with a flexible C_3 chain. Natural DHCs often have phenolic hydroxyl (−OH) groups, with some −OH groups at specific locations substituted with glycosides. The effect of chemical structure, −OH group position, and glycosylation on antioxidant potency of DHCs is still poorly understood.

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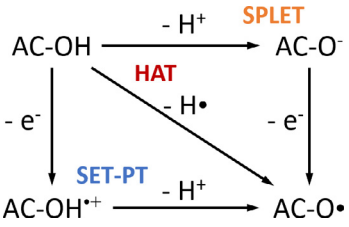

Scheme 1. Mechanisms of antioxidant action.

In this study, the ACs of two DHC aglycones and four glycosylated derivatives were measured under various conditions using 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays. The antioxidant mechanisms of DHC molecules and the effect of glycosylation were determined. In addition, a unique phenomenon that occurred during free radical scavenging by DHCs was observed.

2. Materials and methods

2.1. Chemicals and reagents

Phlorizin (phloretin-2′-*O*-glucoside, P2G), trilobatin (phloretin-4′-*O*-glucoside, P4G), 3-hydroxyphlorizin (3-hydroxyphloretin-2′-*O*-glucoside, HP2G), and sieboldin (3-hydroxyphloretin-4′-*O*-glucoside, HP4G) were extracted and purified from crabapple fruits (*Malus* 'Red Splendor'). Phloretin (P) and 3-hydroxyphloretin (HP) were obtained by the hydrolysis of phlorizin and sieboldin, respectively, as described by Xiao, et al. (2017). Potassium peroxodisulfate ($K_2S_2O_8$), phenol, *p*-cresol, 4-ethylphenol, pyrocatechol, 4-methylcatechol, 4-ethylcatechol, phloroglucinol, phloroacetophenone, phloropropiophenone, 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,2-diphenyl-1-picrylhydrazyl (DPPH), deuterium oxide (D_2O), sodium deuteroxide (NaOD, 40 wt% solution in D_2O), hydrochloric acid, and potassium hydroxide were purchased from J&K Scientific (Beijing, China). Ultra-pure water was prepared using a Millipore Milli-Q system (Darmstadt, Germany). All water used was ultrapure, unless otherwise noted. Methanol and acetone were purchased from Guanghua Sci-Tech Co., Ltd. (Guangdong, China). All solvents were degassed with dry nitrogen to remove dissolved O_2 and CO_2 before use.

2.2. Antioxidant capacity evaluation

The DPPH assay was performed according to the method of Sousa, et al., (2016) with some modifications. It was prepared at 87 μM in solution with 40% methanol-water solution, acetone, or 40% methanol-phosphate buffer (50 mM) with a pH of 2.5, 4.0, 6.0, or 8.0 respectively and used for AC measurement. The pH was monitored using a pH meter (Mettler Toledo, Columbus, Ohio, USA). After adding 20 μL of 5 μM antioxidant compound to 180 μL of DPPH solution, absorbance was measured at 517 nm for acetone mixtures and at 529 nm for methanol-water solutions using an Infinite® 200 Pro (Tecan, Männedorf, Switzerland).

The ABTS assay was performed according to a published method (Re, et al., 1999) with some modifications. Briefly, 7 mM ABTS solution and 2.5 mM potassium peroxodisulfate solution were mixed to produce an ABTS radical cation ($ABTS^{\cdot+}$). This reaction mixture was kept in the dark for 14 h at room temperature before use. The $ABTS^{\cdot+}$ solution was diluted with water, acetone, or phosphate buffer (50 mM) with different pH values. The final absorbance of the $ABTS^{\cdot+}$ solution was 0.90 ± 0.05 at 734 nm, and it was used immediately for AC measurement. After the addition of 100 μL of 5 μM antioxidant compound to 0.9 mL of diluted $ABTS^{\cdot+}$ solution, the mixture was placed in the dark for 1 h. The absorbance was then measured at 723 nm for acetone mixtures and at 734 nm for all other mixtures using a UV-2450 spectrophotometer (Shimadzu, Kyoto, Japan).

A preliminary study determined that the two free radicals were in excess when reacted with 5 μM of antioxidant compound, which guaranteed the full oxidation of DHC compounds by free radicals in the reaction volume.

2.3. Calculation of DHC dissociation constant

The pK_a values for all DHCs in water were determined as described by Ramešová, et al. (2012) with some modifications. Briefly, 50 μM of each DHC compound solution was acidulated to pH 3.0 with hydrochloric acid, then titrated with 0.1 M potassium hydroxide. The titration process was monitored with a precision pH meter at 25 ± 0.5 °C, with the titration flask being purged by nitrogen. The absorbance at 280 nm was recorded from pH 3.0 to pH 10.0. The pK_a was calculated from the pH and measured absorbance values by applying Eq. (1).

$$\mathrm{pH} = \mathrm{p}K_a + \log\frac{(A - A_{min})}{(A_{max} - A)} \quad (1)$$

The A_{max} and A_{min} are the maximum absorbance values measured at the maximum and minimum pH values of the curve, respectively. Plots of $\log[(A - A_{min})/(A_{max} - A)]$ against pH are linear with the intercept equal to pK_a.

The species distribution diagram for DHCs was calculated using Eq. (2). In this equation, $c(DHC^-)$ is the concentration of DHC-anions, K_a is the DHC dissociation constant, $c(H^+)$ is the concentration of hydrogen ions in solution, and$c(DHC)_0$ is the initial concentration of DHC (50 μM).

$$c(DHC^-) = \frac{K_a\, c(DHC)_0}{c(H^+) + K_a} \quad (2)$$

2.4. Nuclear magnetic resonance (NMR) spectroscopy

NMR analysis was performed using a Bruker-500 (Bruker Corporation, Germany) at 500 MHz for ^{1}H NMR spectra. The reference compound tetramethylsilane (TMS) was used as the internal standard, and all samples were dissolved in dimethyl sulfoxide-d_6 (DMSO-d_6).

2.5. Statistical analysis

All data are presented as means ± SE (n = 5). Significant differences were detected by *t*-tests using SPSS 16.0 software (IBM, New York, USA) with $P < 0.05$.

3. Results and discussion

3.1. Antioxidant capacity of DHCs

Six DHC compounds were used in this study, phloretin (P), phlorizin (P2G), trilobatin (P4G), 3-hydroxyphloretin (HP), 3-hydroxyphlorizin (HP2G), and sieboldin (HP4G) (Fig. 1). The AC of all DHCs was evaluated using the DPPH assay in methanol-water solution and ABTS assay in water (Fig. 2A and C). DPPH analysis of the aglycones HP and P revealed that the AC of HP, which has an *o*-dihydroxyl group at the B-ring, was higher than that of P, which has only one −OH group at the B-ring. However, the AC of HP was lower than that of P as determined by ABTS analysis. Glycosylation at the 2′-position significantly reduced the AC of P, but increased that of HP in both assays. Glycosylation at the 4′-position decreased the AC of both P and HP. Generally, the AC of HP2G was highest among all six DHCs in both assays. The AC of P2G was the lowest measured by DPPH assay, whereas the AC of HP4G was the lowest measured by ABTS assay.

It is generally accepted that the *o*-dihydroxyl group chemical structure is conducive to the antioxidant potency of flavonoid (Leopoldini, Russo & Toscano, 2011). Interestingly, the ACs of HP and

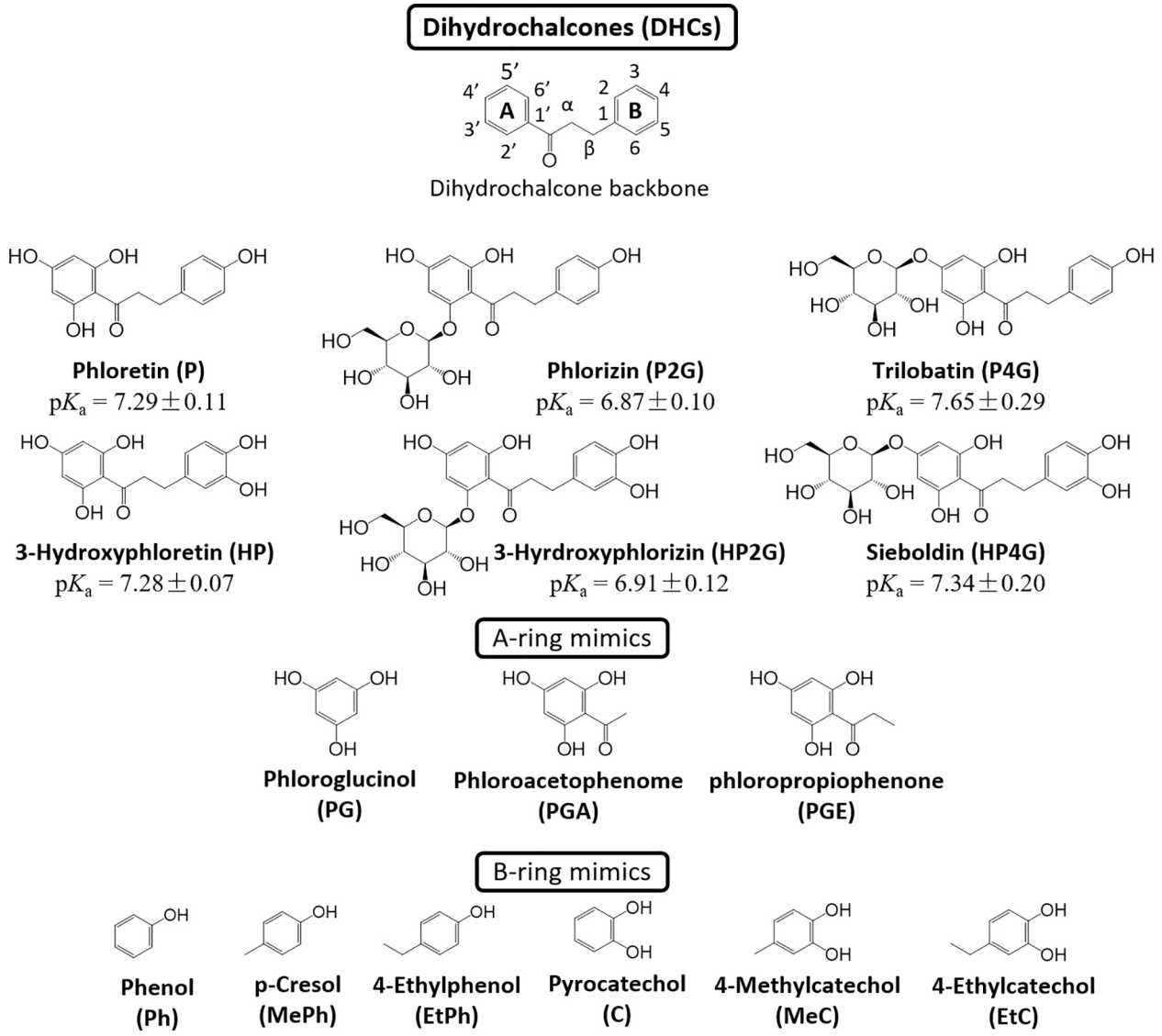

Fig. 1. Chemical structures of dihydrochalcones and phenolic model compounds, and dihydrochalcone pK_a values.

HP4G were lower than P and P4G in the ABTS assay, suggesting that existence of the *o*-dihydroxyl group in DHCs did not always increase antioxidant potency. In addition, *O*-glycosylation always reduced the antioxidant potential of flavonoids (Xiao, 2017). In the present study, glycosylation at the 2′-postion of HP increased the AC. These data suggest that the effects of the *o*-dihydroxyl group and glycosylation on the AC of DHCs may differ from other flavonoids. It is known that the two rings of DHC are not conjugated together like most flavonoids, but are instead linked with a saturated C_3 chain. The flexible molecular structure of DHCs may result in their antioxidant potency different from other flavonoids.

Water and methanol are ionizing and polar solvents, and both support SPLET and HAT mechanisms (Musialik, Kuzmicz, Pawłowski & Litwinienko, 2009). Although SET-PT mechanism is the least preferred from the thermodynamic point of view, but it also may be concurrent in the polar solvents (Stepanić et al., 2013). Therefore, a variety of mechanisms might occur simultaneously during DHC scavenging of $ABTS^{\cdot+}$ and DPPH· in these solvents, and the total AC of DHCs is the result of a combination of multiple mechanisms.

The SPLET mechanism can be eliminated by inhibiting the dissociation of antioxidant compounds in non-ionizing solvents or adding acid into ionizing solvents (Litwinienko & Ingold, 2005; Pyrzynska & Pękal, 2013). Thus we further evaluated the AC of DHC compounds in acetone and acidic solutions to explore the antioxidant mechanisms of DHCs.

3.2. DHC free radical scavenging mechanisms

It is widely accepted that antioxidant compounds scavenge DPPH· primarily through the HAT and SPLET mechanisms (Litwinienko & Ingold, 2004; Musialik & Litwinienko, 2005). The contribution of the SPLET mechanism to AC could be eliminated in acetone or acidic solutions by suppressing dissociation.

The AC of P, P2G, P4G, and HP2G was lower in acetone compared to methanol-water solution for the DPPH assay (Fig. 2A). With the exception of P2G, all other compounds exhibited reduced AC in methanol-water solution with different pH values due to increased solution acidity (Fig. 2B). Moreover, the ACs in pH 2.5 solution were similar to those in acetone. Meanwhile, the AC of HP and HP4G was inhibited in acidic methanol-solution, but not in acetone (Fig. 2A and B). A previous study reported that acetone was more conducive to the HAT mechanism and kinetics compared to methanol-water solution (Litwinienko & Ingold, 2007; Supplementary Table 1). Therefore, it is possible that the equilibrium constants of the reactions of HP and HP4G with DPPH· were greater in acetone than in methanol-water solution. As a result, these two compounds scavenged more DPPH· through the HAT

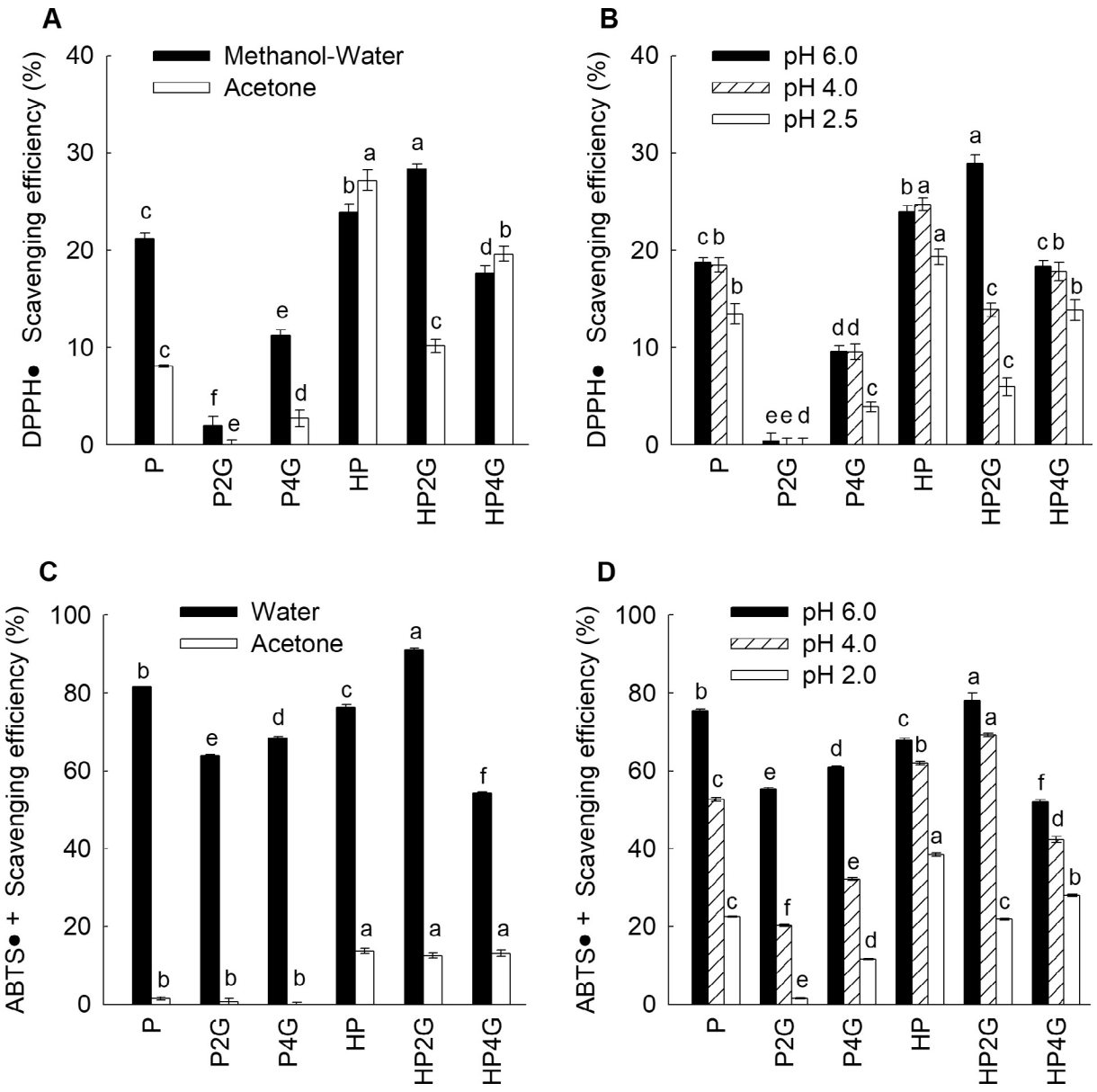

Fig. 2. Antioxidant capacity of dihydrochalcones evaluated using DPPH (A & B) and ABTS (C & D) assays in different solutions. Data are shown as means ± SE (n = 5). Names of the dihydrochalcone compounds were presented in Fig. 1. Different small letters indicate significant difference ($P < 0.05$) among compounds in the same solution.

mechanism in acetone, which compensated for the effect of SPLET suppression on the AC.

Thus the AC in acetone and methanol-water solution at pH 2.5 (Fig. 2A and B) mainly reflects the capacity of DHCs scavenging DPPH· by the HAT mechanism. The AC of DHCs with an *o*-dihydroxyl at the B-ring was higher than that of DHCs with only one −OH group when the chemical structures of the A-ring were the same, indicating that the *o*-dihydroxyl group at the B-ring is conducive to the HAT mechanism. It is reported that the *o*-dihydroxyl group forms intramolecular hydrogen bond, even after H-atom or proton abstraction (Leopoldini et al., 2011).

Glycosylation at the A-ring reduced the AC of DHC through the HAT mechanism, particularly glycosylation at the 2′-position (Fig. 2A and B). This suggests that the 2′-OH is crucial for the HAT mechanism. However, computational investigation showed that the BDE of the 2′-OH was the highest among all the −OH groups of P in water or methanol (Mendes et al., 2018). Since the ketone carbonyl and the A-ring are linked by an σ-bond in the P molecule, the ketone carbonyl would form an intramolecular hydrogen bond with either the 2′- or 6′-OH. As a result, P molecule would become planar, making it easier to donate an H-atom from the other −OH group that did not form an intramolecular hydrogen bond with the ketone carbonyl (Leopoldini et al., 2011). Glycosylation at the 2′-postion changes the symmetry of the P molecule, and a ketone carbonyl could only form an intramolecular hydrogen bond with the 6′-OH. Such a chemical structure would impede H-atom donation from the 6′-OH and inhibit the HAT mechanism. Glycosylation at the 4′-position of the A-ring does not change the symmetry of the P molecule, and the BDE of the 4′-OH implies that this −OH location is not favorable for the HAT reaction (Mendes et al., 2018). Hence, glycosylation at the 4′-position might affect the AC through steric hindrance of glucoside. Because DHC A-rings and B-rings are not conjugated, the effect of glycosylation at the A-ring of HP was the same as that of P.

On the B-ring of P molecule, the 4-OH had the lowest BDE (Mendes et al., 2018). However, in this study P2G hardly scavenged free radicals through the HAT mechanism in acetone or pH 2.5 methanol-water solution, demonstrating that H-atom transfer is difficult from this

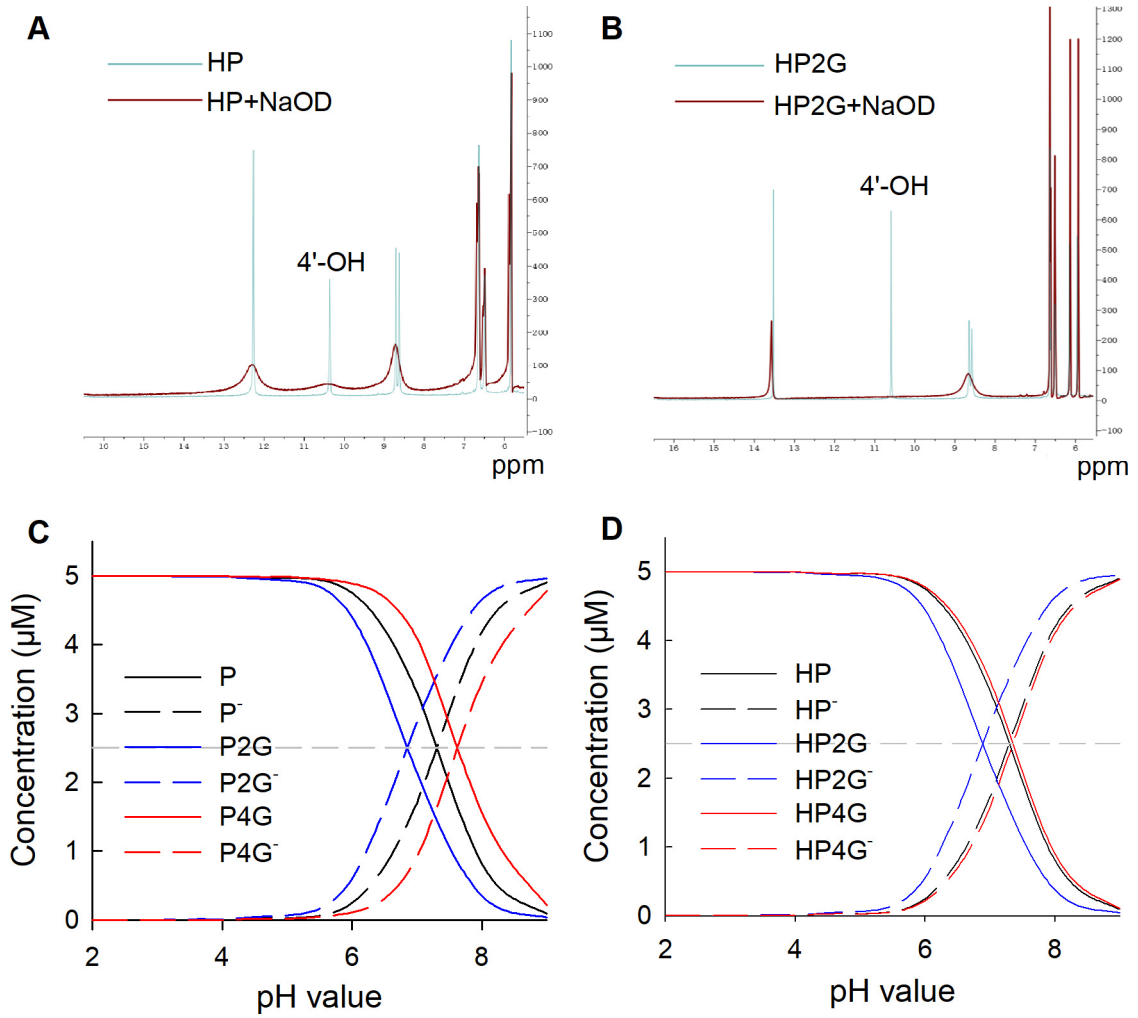

Fig. 3. Panels A & B: ^{1}H NMR spectra of HP (A) and HP2G (B) in DMSO-$d_6$before and after addition of 0.2 μmol NaOD. Panels C &D: species distribution diagram for P, P2G, and P4G (C), and HP, HP2G, and HP4G (D). Names of the dihydrochalcone compounds were presented in Fig. 1.

location.

In the ABTS assay, the AC of all compounds was lower in acetone than in water and decreased with increasing acidity (Fig. 2C and D). These results suggest that the SPLET mechanism was involved in the scavenging of $ABTS^{\cdot+}$ by DHCs in water. DHCs with only one −OH group on the B-ring exhibited almost no AC in acetone, and those with an *o*-dihydroxyl showed similar AC. Thus the AC of DHCs scavenging $ABTS^{\cdot+}$ in acetone did not relate to the chemical structure of the A-ring, and the HAT mechanism only occurred when the *o*-hydroxyl group was present on the B-ring.

The AC of all six DHCs in pH 2.0 solution differed from that in acetone (Fig. 2C and D). In pH 2.0 solution, the AC of HP and its derivatives was higher than the counterpart P and its derivatives. Glycosylation at the A-ring, especially at the 2′-position, decreased the AC of the two aglycones. P2G showed almost no AC, but P and P4G, which have no *o*-dihydroxyl on the B-ring, exhibited AC in pH 2.0 solution. Compared to DPPH·, which more easily reacts with antioxidants through the HAT mechanism, $ABTS^{\cdot+}$ scavenging generally involves the SET-PT mechanism (Köksal, Gülçin, Beyza, Sarikaya & Bursal, 2009). The polarity of water is stronger and more convenient for the SET mechanism compared to acetone (Stepanić et al., 2013). Therefore, we speculated that in addition to the SPLET and HAT mechanisms, the SET mechanism also occurred during DHC scavenging of $ABTS^{\cdot+}$ in pH 2.0 solution. Previous studies reported that the IP value of P2G was higher than that of P (Bentes, Borges, Monteiro, De Macedo & Alves, 2011; Mendes et al., 2018). Higher IP value is not conducive to the occurrence of the SET-PT mechanism, which is consistent with P2G showing almost no AC in pH 2.0 solution. In addition, the SET mechanism was inhibited more by glycosylation at the 2′-position than at the 4′-position. Since the chemical structure of HP's A-ring is the same as that of P, the effect of glycosylation on the AC of P was the same as that of HP.

3.3. A potentially unique DHC free radical scavenging mechanism

All DHC polyphenolic hydroxyl groups can dissociate. The first step of dissociation produces the most anions and is the most important for the SPLET mechanism. For P and P2G molecules, the first −OH group to dissociate is at the 4′-position of the A-ring (Xiao et al., 2017). ^{1}H NMR spectra for HP and HP2G showed that the peaks of all −OH groups broadened and peak areas decreased with the addition of NaOD. Moreover, the 4′-OH group peaks for HP (chemical shift, 10.27 ppm) and HP2G (chemical shift, 10.60 ppm) broadened greatly and even disappeared compared to peaks of the other −OH groups (Fig. 3A and B). A similar phenomenon was observed with addition of D_2O (Supplementary Figs. S1 and S2). Therefore, compared to other −OH groups, the 4′-OH of HP and HP2G more easily exchanged its hydrogen with deuterium, and is firstly dissociated as the 4′-OH of P and P2G.

According the Gibbs–Helmholtz equation and the definition of PA, the dissociation constant (pK_a) which directly reflects the dissociation ability of a compound in water solution, was positively correlated with PA value (Rossini & Knapp, 2016). The pK_a values of the two aglycones

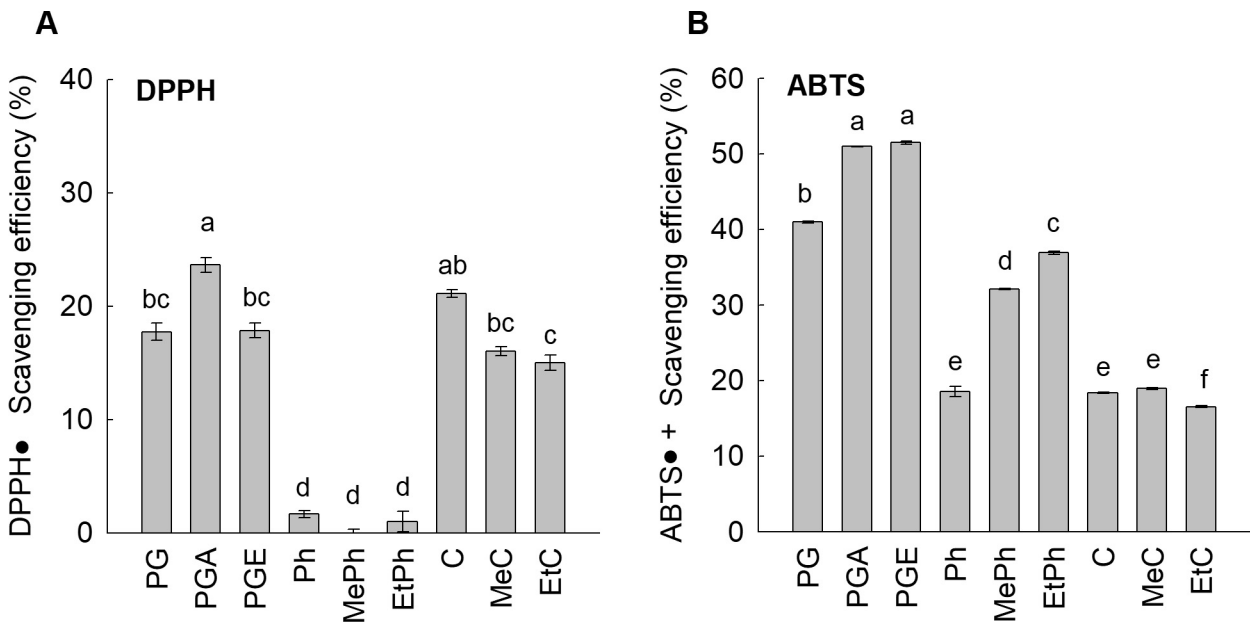

Fig. 4. Antioxidant capacity of mimics determined by DPPH and ABTS assays in methanol-water solution and water, respectively. Names of mimics are presented in Fig. 1. Different small letters indicate significant difference ($P < 0.05$) among compounds.

were similar (Fig. 1, Supplementary Fig. S3), indicating that hydroxylation of the B-ring did not affect dissociation of the 4′-OH of the A-ring since the two rings are not conjugated. P2G and HP2G pK_a values were the same and lower than those found for their aglycones, suggesting that glycosylation at the 2′-position promoted dissociation of the 4′-OH. Glycosylation of the 4′-OH caused the other −OH group, whose dissociation ability was weaker than the 4′-OH, to dissociate first. Therefore, the pK_a values of P4G and HP4G increased compared to the aglycones (Fig. 1). Decreased dissociation ability might explain why P4G and HP4G had lower AC compared to their aglycones. P2G had higher dissociation ability but lower AC than its aglycone, P, indicating that the second step of the SPLET mechanism (electron transfer) might be important for AC in water-based solution.

For P and P2G, the first dissociation occurred at the 4′-OH. The negative charge of anion that was formed after dissociation was present on the A-ring, and therefore electron transfer with free radicals should occur from the A-ring of P and P2G. The reaction of DPPH· with the mimic compounds, which have only one −OH group (Fig. 4A), implies that the AC of P and P2G is mainly attributed to the A-ring in methanol-water solution. Because DPPH· is sensitive to steric hindrance (Gülçin, 2012; Yang et al., 2018), the steric hindrance of glucoside at the 2′-postion of P2G molecules would block electron transfer from the A-ring to DPPH·. As a result, the AC of P2G was very low in methanol-water solution.

Similar to P2G, HP2G also dissociated through the 4′-OH group and had the glycosylated substituent at the A-ring. However, the AC of HP2G was not negatively affected by the steric hindrance (Fig. 2). Therefore, DPPH· might react with HP2G through the *o*-dihydroxyl on the B-ring. Glycosylation caused the 4′-OH on HP2G's A-ring to dissociate easier than the 4′-OH on HP, which prompted the B-ring to scavenge DPPH·. Indeed, when DHCs and DPPH· were reacted under alkaline conditions, causing more DHC molecules to dissociate, the AC of P and its derivatives did not increase, but that of HP and HP4G did increase (Supplementary Fig. S4). This further demonstrates that the dissociation of DHC molecules at the A-ring could improve the AC when the B-ring contains *o*-dihydroxyl instead of a singular hydroxyl group. We speculate that this improvement in AC was achieved through electron transfer from the dissociated A-ring to the oxidized B-ring. The AC of HP2G was the highest among all DHCs for the ABTS assay in water, which also supports this opinion. Future studies are required to determine if this phenomenon is part of the SPLET mechanism.

In addition, all mimics exhibited scavenging capacity for $ABTS^{\cdot+}$, indicating that the AC of DHCs might be attributed to both the A-ring and the B-ring. Moreover, the AC of mimics with an *o*-dihydroxyl group were lower than those with only one −OH group, suggesting that the *o*-dihydroxyl group was not an efficient chemical structure for scavenging $ABTS^{\cdot+}$ in water solution. Therefore, HP and HP4G exhibited lower AC than P and P4G in water, respectively (Fig. 2C and D).

4. Conclusion

Evaluation of the AC of DHCs showed that HP2G had the highest antioxidant potency in water-based solution. This could be due to glycosylation at the A-ring of HP2G molecules, which increased dissociation of the 4′-OH and increased scavenging of free radicals by the B-ring. Furthermore, the *o*-dihydroxyl at the B-ring and the 2′-OH of DHC molecules increased antioxidant potency by promoting the HAT mechanism. The 2′-OH of DHCs was conducive to free radical scavenging through the SET mechanism. These data show that DHCs are excellent antioxidants that can scavenge free radicals through a variety of mechanisms.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodchem.2018.09.135.

References

Amić, A., Lučić, B., Stepanić, V., Marković, Z., Marković, S., Marković, J. M. D., & Amić, D. (2017). Free radical scavenging potency of quercetin catecholic colonic metabolites: Thermodynamics of $2H^+/2e^-$ processes. *Food Chemistry, 218*, 144–151.

Amic, A., Markovic, Z., Markovic, J. M., Stepanic, V., Lucic, B., & Amic, D. (2014). Towards an improved prediction of the free radical scavenging potency of flavonoids:

The significance of double PCET mechanisms. *Food Chemistry,* 578–585.
Bentes, A., Borges, R. S., Monteiro, W. R., De Macedo, L., & Alves, C. N. (2011). Structure of dihydrochalcones and related derivatives and their scavenging and antioxidant activity against oxygen and nitrogen radical species. *Molecules, 16*(2), 1749–1760.
Bordenave, N., Hamaker, B. R., & Ferruzzi, M. G. (2014). Nature and consequences of non-covalent interactions between flavonoids and macronutrients in foods. *Food & Function, 5*(1), 18–34.
Chen, C., Zhang, D., Wang, Y., Li, P., & Ma, F. (2012). Effects of fruit bagging on the contents of phenolic compounds in the peel and flesh of 'Golden Delicious', 'Red Delicious', and 'Royal Gala' apples. *Scientia Horticulturae,* 68–73.
Forman, H. J., Davies, K. J., & Ursini, F. (2014). How do nutritional antioxidants really work: Nucleophilic tone and para-hormesis versus free radical scavenging in vivo. *Free Radical Biology and Medicine, 66*, 24–35.
Galano, A., Mazzone, G., Alvarez-Diduk, R., Marino, T., Alvarez-Idaboy, J. R., & Russo, N. (2016). Food antioxidants: Chemical insights at the molecular level. *Annual Review of Food Science and Technology, 7*, 335–352.
Ghiselli, A., Serafini, M., Natella, F., & Scaccini, C. (2000). Total antioxidant capacity as a tool to assess redox status: Critical view and experimental data. *Free Radical Biology and Medicine, 29*(11), 1106–1114.
Gomes de Moura, C. F., & Ribeiro, D. A. (2017). Are food compounds able to modulate noxious activities induced by cadmium exposure? *Critical Reviews in Food Science and Nutrition, 57*(3), 632–636.
Gülçin, İ. (2012). Antioxidant activity of food constituents: An overview. *Archives of Toxicology, 86*(3), 345–391.
Klein, E., Rimarčík, J., Senajová, E., Vagánek, A., & Lengyel, J. (2016). Deprotonation of flavonoids severely alters the thermodynamics of the hydrogen atom transfer. *Computational and Theoretical Chemistry, 1085*, 7–17.
Köksal, E., Gülçin, İ., Beyza, S., Sarikaya, Ö., & Bursal, E. (2009). In vitro antioxidant activity of silymarin. *Journal of Enzyme Inhibition and Medicinal Chemistry, 24*(2), 395–405.
Leopoldini, M., Russo, N., & Toscano, M. (2011). The molecular basis of working mechanism of natural polyphenolic antioxidants. *Food Chemistry, 125*(2), 288–306.
Lin, Y. P., Hsu, F. L., Chen, C. S., Chern, J. W., & Lee, M. H. (2007). Constituents from the Formosan apple reduce tyrosinase activity in human epidermal melanocytes. *Phytochemistry, 68*(8), 1189–1199.
Litwinienko, G., & Ingold, K. U. (2003). Abnormal solvent effects on hydrogen atom abstractions. 1. The reactions of phenols with 2,2-diphenyl-1-picrylhydrazyl (dpph•) in alcohols. *Journal of Organic Chemistry, 68*(9), 3433–3438.
Litwinienko, G., & Ingold, K. U. (2004). Abnormal solvent effects on hydrogen atom abstraction. 2. Resolution of the curcumin antioxidant controversy. The role of sequential proton loss electron transfer. *Journal of Organic Chemistry, 69*(18), 5888–5896.
Litwinienko, G., & Ingold, K. U. (2005). Abnormal solvent effects on hydrogen atom abstraction. 3. Novel kinetics in sequential proton loss electron transfer chemistry. *Journal of Organic Chemistry, 70*(22), 8982–8990.
Litwinienko, G., & Ingold, K. U. (2007). Solvent effects on the rates and mechanisms of reaction of phenols with free radicals. *Accounts of Chemical Research, 40*(3), 222–230.
Mazzone, G., Galano, A., Alvarez-Idaboy, J. R., & Russo, N. (2016). Coumarin–chalcone hybrids as peroxyl radical scavengers: Kinetics and mechanisms. *Journal of Chemical Information and Modeling, 56*(4), 662–670.
Mazzone, G., Malaj, N., Galano, A., Russo, N., & Toscano, M. (2015). Antioxidant properties of several coumarin–chalcone hybrids from theoretical insights. *RSC Advances, 5*(1), 565–575.
Mendes, R. A., e Silva, B. L., Takeara, R., Freitas, R. G., Brown, A., & de Souza, G. L. (2018). Probing the antioxidant potential of phloretin and phlorizin through a computational investigation. *Journal of Molecular Modeling, 24*(4), 101.
Musialik, M., Kuzmicz, R., Pawłowski, T., & Litwinienko, G. (2009). Acidity of hydroxyl groups: An overlooked influence on antiradical properties of flavonoids. *Journal of Organic Chemistry, 74*(7), 2699–2709.
Musialik, M., & Litwinienko, G. (2005). Scavenging of dpph• radicals by vitamin E is accelerated by its partial ionization: The role of sequential proton loss electron transfer. *Organic Letters, 7*(22), 4951–4954.
Niki, E. (2014). Role of vitamin E as a lipid-soluble peroxyl radical scavenger: In vitro and in vivo evidence. *Free Radical Biology and Medicine, 66*, 3–12.
Pisoschi, A. M., & Pop, A. (2015). The role of antioxidants in the chemistry of oxidative stress: A review. *European Journal of Medicinal Chemistry, 97*, 55–74.
Prior, R. L., Wu, X., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry, 53*(10), 4290–4302.
Pyrzynska, K., & Pękal, A. (2013). Application of free radical diphenylpicrylhydrazyl (DPPH) to estimate the antioxidant capacity of food samples. *Analytical Methods, 5*(17), 4288–4295.
Ramešová, S., Sokolová, R., Degano, I., Bulíčková, J., Zabka, J., & Gál, M. (2012). On the stability of the bioactive flavonoids quercetin and luteolin under oxygen-free conditions. *Analytical and Bioanalytical Chemistry, 402*(2), 975–982.
Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine, 26*(9–10), 1231–1237.
Rossini, E., & Knapp, E. W. (2016). Proton solvation in protic and aprotic solvents. *Journal of Computational Chemistry, 37*(12), 1082–1091.
Shadnia, H., & Wright, J. S. (2008). Understanding the toxicity of phenols: Using quantitative structure-activity relationship and enthalpy changes to discriminate between possible mechanisms. *Chemical Research in Toxicology, 21*(6), 1197–1204.
Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods, 18*(12), 757–781.
Sousa, A., Araújo, P., Azevedo, J., Cruz, L., Fernandes, I., Mateus, N., & de Freitas, V. (2016). Antioxidant and antiproliferative properties of 3-deoxyanthocyanidins. *Food Chemistry, 192*, 142–148.
Stepanić, V., Trošelj, K. G., Lučić, B., Marković, Z., & Amić, D. (2013). Bond dissociation free energy as a general parameter for flavonoid radical scavenging activity. *Food Chemistry, 141*(2), 1562–1570.
Tsao, R., Yang, R. S., Young, J. C., & Zhu, H. (2003). Polyphenolic profiles in eight apple cultivars using high-performance liquid chromatography (HPLC). *Journal of Agricultural and Food Chemistry, 51*(21), 6347–6353.
Vagánek, A., Rimarčík, J., Dropková, K., Lengyel, J., & Klein, E. (2014). Reaction enthalpies of OH bonds splitting-off in flavonoids: The role of non-polar and polar solvent. *Computational and Theoretical Chemistry, 1050*, 31–38.
Xiao, J. (2017). Dietary flavonoid aglycones and their glycosides: Which show better biological significance? *Critical Reviews in Food Science and Nutrition, 57*(9), 1874–1905.
Xiao, Z., Zhang, Y., Xian, C., Wang, Y., Chen, W., Xu, Q., Li, & Ma, F. (2017). Extraction, identification, and antioxidant and anticancer tests of seven dihydrochalcones from *Malus*, 'Red Splendor' fruit. *Food Chemistry, 231*, 324–331.
Yang, H., Xue, X., Li, H., Apandi, S. N., Tay-Chan, S. C., Ong, S. P., & Tian, E. F. (2018). The relative antioxidant activity and steric structure of green tea catechins–A kinetic approach. *Food Chemistry, 257*, 399–405.