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Quantitative Structure-Activity Relationship (QSAR) of Antioxidative Anthocyanidins and Their Glycosides

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Abstract The quantitative structure-activity relationships (QSAR) study of antioxidative anthocyanidins and their glycosides were evaluated using 4 different assays of Trolox equivalent antioxidant capacity (TEAC), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and peroxynitrite radical ($ONOO^-$) scavenging with TSAR software. Four models were developed with significant predictive values (r^2 and p value), which indicated that the antioxidant activities were mainly governed by the 3-dimensional structural energy (torsional energy), constitutional properties (the number of hydroxyl and methyl groups), and electrostatic properties (heat of formation, and dipole, quadrupole, and octupole components). This QSAR approach could contribute to a better understanding of structural properties of anthocyanidins and their glycosides that are responsible for their antioxidant activities. It might also be useful in predicting the antioxidant activities of other anthocyanins.

Keywords: anthocyanidin, anthocyanin, antioxidative, radical-scavenging, quantitative structure-activity relationship (QSAR)

Introduction

Anthocyanins are widely distributed in many flowers, fruits, vegetables, and grains, and have blue, purple, and red colors. The contents of anthocyanins in fruits and vegetables have been estimated to range from 19 to 21,600 mg/kg (1). They are thought to have beneficial effects on human health through reducing the risk of cardiovascular disease and improving vision (2,3). Previous studies on the biological functions of anthocyanins have consistently reported antimutagenic, anti-inflammatory, antioxidative, anti-ulcer, anticancer, vasoprotective, and neuroprotective effects (4-11). The broad range of biological activities in anthocyanins are often linked to their potent antioxidative properties (12,13). Recognition of these health benefits has led to the discovery of new anthocyanins, and to the elaboration of dietary supplements containing them. Nonetheless, anthocyanin molecules that exhibit remarkable activity and stability to pH change, and heat and light exposure, are still under consideration.

Reactive oxygen species (ROS), including the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radical ($OH^{\cdot}$), singlet oxygen ($^1O_2$), nitric oxide ($NO^{\cdot}$), and peroxynitrite radical ($ONOO^-$), are generated as byproducts of normal metabolism. ROS are essential for the host defense system; however, increased ROS levels lead to oxidative stress, biochemical and physiological lesions, and eventually cell death (14). Cumulative oxidative damage leads to numerous disorders and diseases (15). Thus, the prevention of oxidative stress caused by natural antioxidants, such as anthocyanins, could be of great importance.

The antioxidative and free radical-scavenging activities of anthocyanins have been measured by using extracts from various natural products and pure compounds (6,16). These antioxidative and free radical-scavenging activities are closely related to physicochemical properties that are attributable to the molecular structures of compounds (17). Structure-activity relationship (SAR) and quantitative structure-activity relationship (QSAR) studies are useful tools for investigating the relationships between the chemical structures of compounds and their bioactivity (18-21). Many authors have attempted to investigate the SARs/QSARs relating to the antioxidative and free radical-scavenging activities of phenolic antioxidants (17,22-24). Anthocyanins have been partly explored in these studies as a type of flavonoids. However, they differ from other members of the flavonoid group due to their ability to form flavylium (2-phenylbenzopyrylium) cations (6).

Recently, a study on the SAR concerning radical-scavenging activities of anthocyanins (glycoside forms) has been reported (25), but studies on the QSAR have not been attempted. In addition, many studies on the bioactivities of anthocyanins have focused on the aglycone forms (anthocyanidins), particularly cyanidine and delphinidin, which are known to be more bioactive than anthocyanins (5,26). In the current study, not only anthocyanidins but also their glycosides were included in the QSAR model development, because these forms are commonly found in nature, and their intact glycosides can be detected in the circulatory system of the body (27,28). From our previous studies, we found that the protective effect of anthocyanin-rich extract from bilberry against 5-fluorouracil-induced myelotoxicity could be explained in part as a result of its antioxidative activity. According to our other study, the cytoprotective effect of anthocyanins against doxorubicin-induced toxicity in H9c2 cardiomyocytes was related with their antioxidant activities (29,30). Thus, the following study to explore quantitative relationship between their structures and antioxidative activities would be necessary.

The objective of this study was to develop a reliable QSAR model using multiple linear regressions to predict

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the antioxidant activities of other anthocyanidins and their glycosides. Six anthocyanidins as aglycones and 7 anthocyanins as glycosides, each of which contained a sugar unit at C3 position in the C ring (glucose or galactose), were selected for this study.

Materials and Methods

Compounds included Six anthocyanidins (cyanidin chloride, delphinidin chloride, malvidin chloride, pelagonidin chloride, peonidin chloride, and petunidin chloride) and 7 anthocyanins (cyanidin 3-*O*-β-glucopyranoside chloride, delphinidin 3-*O*-β-glucopyranoside chloride, malvidin 3-*O*-β-glucopyranoside chloride, pelagonidin 3-*O*-β-glucopyranoside chloride, peonidin 3-*O*-β-glucopyranoside chloride, petunidin 3-*O*-β-glucopyranoside chloride, and cyanidin 3-*O*-β-galactopyranoside chloride) included in this study were obtained from Polyphenols Laboratories AS (Sandnes, Norway) and their structures were shown in Table 1.

Literature data extracted Antioxidative activities including Trolox equivalent antioxidative capacity (TEAC), and scavenging of superoxide radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), and peroxynitrite radical ($ONOO^-$) were examined as presented in our previous article (29), and the data were extracted for this QSAR study.

Molecular descriptors The 3-dimensional (3-D) structures of 6 anthocyanidins and 7 anthocyanins were generated using the Corina program, by inputting the 2-D structures drawn by Chem Draw, and their energy was optimized and calculated using the COSMIC function in TSAR™ version 3.3 software (31). The molecular descriptors were calculated using TSAR. The electrostatic properties were generated by the VAMP AM1 semiempirical molecular orbital package added to TSAR. The descriptors were obtained for the whole molecules and TSAR calculated the following: the 3-D structural energy (bond, angle, torsional, electrostatic, Van der Waals, and total energy); molecular mass, molecular surface area, and volume; moments of inertia [moment 1, 2, 3 (size, length), and ellipsoidal volume]; dipole moments (total, and x, y, z components); lipole moments (total, and x, y, z components); log P; molecular connectivity indices (Chi and ChiV indices); molecular shape indices (Kappa and Kalpha indices); topological indices (Wiener, Randic, and Balaban indices); electrotopological-state indices [total energy, electronic energy, nuclear repulsion energy, accessible surface area, overall atomic charge, mean polarizability, heat of formation (HF), symmetry point group, highest occupied molecular orbital (HOMO), lowest unoccupied molecular orbital (LUMO), ionization potential, total dipole moment, polarizability components, dipole components, quadrupole components, and octupole components]; number of defined atoms (carbons, oxygens, and so on); number of defined groups (hydroxyl, methyl, and so on).

QSAR model development Stepwise multiple linear regressions were performed using TSAR™ version 3.3 software (31). Correlation coefficients between the activity and the descriptor, or descriptors were determined by correlation analysis (SAS Institute Inc., Cary, NC, USA). The selected descriptors were obtained by discarding highly intercorrelated ($r>0.8$) descriptors and selecting those that appeared with higher frequency in previous models. The selected descriptors were then used to build the QSAR model. Final models with no more than 3 descriptors, a squared correlation coefficient (r^2) higher than 0.7, and cross-validated r^2 (q^2) greater than 0.6, were chosen. The quality of the regression models was evaluated by r^2, q^2, and probability (p). Leave-one-out cross-validation (q^2) was applied, which indicated the predictive power of the multiple regression equation.

Results and Discussion

Antioxidant activities of anthocyanins and their glucosides Descriptions of the anthocyanidins and their glycosides are presented in Table 1. The anthocyanidins and their glycosides all showed antioxidant activities when measured by the 4 different methods, with TEAC values of 1.44-3.97 in the TEAC assay, and IC_{50} values (μM) of 15.55-362.47 in the $O_2^{\bullet -}$ scavenging assay, 10.69-31.49 in the H_2O_2 scavenging assay, and 0.84-6.31 in the $ONOO^-$ scavenging assay (Table 1). The IC_{50} values in the $ONOO^-$ scavenging assay were the lowest among the 4 assays. In the TEAC and $ONOO^-$ scavenging assays, the anthocyanins showed higher activities than those of the anthocyanidins as a whole, but they had lower activity than that of anthocyanidins in the $O_2^{\bullet -}$ scavenging activity. Cyanidin, delphinidin, and petunidin displayed high activities in all 4 assays with delphinidin showing the highest activities among the aglycones in all of the assays. Considering that concentration of anthocyanins and their metabolites in the human serum reached levels of 197.3-986.1 nmol/L after orally consumed chokeberry extract containing 1.3 g cyanidine 3-glycosides (32), anthocyanins, which demonstrated IC_{50} values of 0.84-362.47 in this study, might be effective in physiological condition.

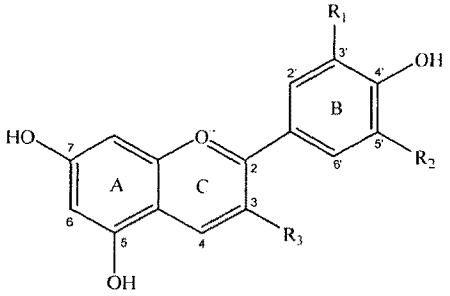
These results differed from those of previous reports on the antioxidative effects of flavonoids, which showed that aglycones were generally more active than glycosides owing to the steric hindrance of sugars in the molecules (17,33). By contrast, several studies using oxygen radical absorbance capacity (ORAC) or 2,2-diphenyl-1-picrylhydrazyl (DPPH) assays, which were highly correlated with the TEAC assay for anthocyanins, reported that the antioxidative activities of several glycosides were superior to those of aglycones, depending on the kind of sugar present (34,35). This indicated that the antioxidative activities of anthocyanidins and their glycosides depend on the kinds of aglycones or glycosides present, the assay method used, and the assay conditions, even when the same method is adopted.

An important structural property of flavonoids is hydroxylation of the ring structure, as well as glycosylation and methylation. In particular, the NH in the B ring is important for anthocyanins (23,36). In the case of aglycones, the most effective results in the assays were those for delphinidin with 3', 4', 5'-OH, cyanidin with 3', 4'-OH, and petunidin with 4', 5'-OH and 3'-OCH_3 groups in the B ring. Delphinidin with 3 OH groups in the B ring showed the highest activity among the anthocyanidins in all 4 assays. Petunidin substituted with a methyl group at the 3'-*O*-position showed decreased activities compared to

Table 1. The chemical structures of anthocyanins and their antioxidant activities

	Compounds	Substituents			Antioxidant activities[1]			
		R_1	R_2	R_3	TEAC value[2]	IC_{50}[3]		
						$O_2^{\cdot-}$	H_2O_2	$ONOO^-$
1	Cyanidin	OH	H	OH	2.63	24.41	11.63	1.66
2	Delphinidin	OH	OH	OH	3.16	15.55	10.69	1.65
3	Malvidin	OCH_3	OCH_3	OH	1.96	37.39	31.49	3.44
4	Pelargonidin	H	H	OH	1.46	36.54	18.85	5.01
5	Peonidin	OCH_3	H	OH	1.44	44.25	29.40	6.31
6	Petunidin	OCH_3	OH	OH	1.89	16.97	13.81	2.20
7	Cyanidin-3-*O*-glucoside	OH	H	*O*-β-glucose	3.63	362.47	12.70	0.87
8	Delphinidin-3-*O*-glucoside	OH	OH	*O*-β-glucose	2.85	243.02	16.12	0.94
9	Malvidin-3-*O*-glucoside	OCH_3	OCH_3	*O*-β-glucose	2.98	113.71	18.22	0.96
10	Pelargonidin-3-*O*-glucoside	H	H	*O*-β-glucose	2.44	71.19	20.36	3.79
11	Peonidin-3-*O*-glucoside	OCH_3	H	*O*-β-glucose	2.86	104.90	19.86	3.67
12	Petunidin-3-*O*-glucoside	OCH_3	OH	*O*-β-glucose	3.39	54.84	10.75	0.87
13	Cyanidin-3-*O*-galactoside	OH	H	*O*-β-galactose	3.97	164.76	11.96	0.84

[1]Data on antioxidant activities are extracted from our previous study (ref. 29). Ascorbic acid was used as a positive control for antioxidant activities, representing. TEAC value of 0.99, and IC_{50} of 260.69 ($O_2^{\cdot-}$), 46.26 (H_2O_2), and 0.88 ($ONOO^-$) in radical-scavenging assays.
[2]Defined as the concentration of standard Trolox solution with equivalent antioxidant potential to a 1 μM concentration of the antioxidant compound in the present study.
[3]Defined as the μM concentration of the compound required to inhibit radical production by 50%.



delphinidin without a methoxyl group. These results were consistent with Rahman's report (25) on superoxide radical- and peroxynitrite-scavenging activities of 15 purified bilberry anthocyanins in that the number of free hydroxyl groups and *O*-methylation on the B-ring determines the reactivity of anthocyanins.

QSAR models for anthocyanidins and their glycosides

A list of the calculated molecular descriptors for anthocyanins used in the multiple regression analysis is presented in Table 2. The best QSAR models for each activity are described by the equations below.

$$TEAC = -0.0050\ HF + 0.0051\ OZZ + 2.5036 \quad (1)$$

(n=13, r=0.858, r^2=0.736, q^2 (CV)=0. 710, s=0.451, F=13.907, p<0.001)

$$O_2^{\cdot -} = 15.714\ TE - 1.4125\ QYY + 84.970 \quad (2)$$

(n=13, r=0.938, r^2=0.880, q^2 (CV)=0.811, s=39.051, F=36.775, p<0.001)

$$H_2O_2 = 8.9649\ NM - 1.7579\ DX + 0.4153\ QXY + 6.6193 \quad (3)$$

(n=13, r=0.895, r^2=0.802, q^2 (CV)=0.680, s=3.479, F=12.115, p<0.001)

$$ONOO^- = -0.9038\ NH + 0.2080\ QYZ + 8.3954 \quad (4)$$

(n=13, r=0.895, r^2=0.802, q^2 (CV)=0.704, s=0.880, F=20.235, p<0.001)

In these equations, n represents the number of compounds, r represents the correlation coefficient, r^2 represents the squared correlation coefficient, q^2 represents the cross-validated r^2, s represents the standard error of prediction, and F represents the F-ratio.

These models exhibited high prediction values, as can be seen from the r^2, q^2, and significant p values. Each of the descriptors included in the equations was significant at p<0.05 (Eq. 1) or 0.01 (Eq. 2, 3, 4) as given in t-probability (data not shown). Equation 1 shows the HF and octupole ZZY (OZZ) as important descriptors of total antioxidant activity (TEAC assay) of anthocyanidins and their glycosides. The TEAC increases as the HF decreases, while the OZZ increases. By contrast, Eq. 2 describes the torsional energy (TE) and quadrupole YY (QYY) as the important descriptors of the $O_2^{\cdot -}$ scavenging activity of anthocyanidins and their glycosides. The $O_2^{\cdot -}$ scavenging activity increases as the TE increases, while the QYY decreases. Equation 3 describes the number of methyl groups (NM), dipole X (DX), and quadrupole XY (QXY) as the important descriptors of the H_2O_2 scavenging activity of anthocyanidins and their glycosides. The H_2O_2 scavenging activity increases as the NM and QXY increase, while the DX decreases. Equation 4 shows the number of hydroxyl groups (NH) and quadrupole YZ (QYZ) as the important descriptors of the $ONOO^-$ scavenging activity of anthocyanidins and their glycosides. The $ONOO^-$ scavenging activity increases as the NH decreases, while the QYZ increases.

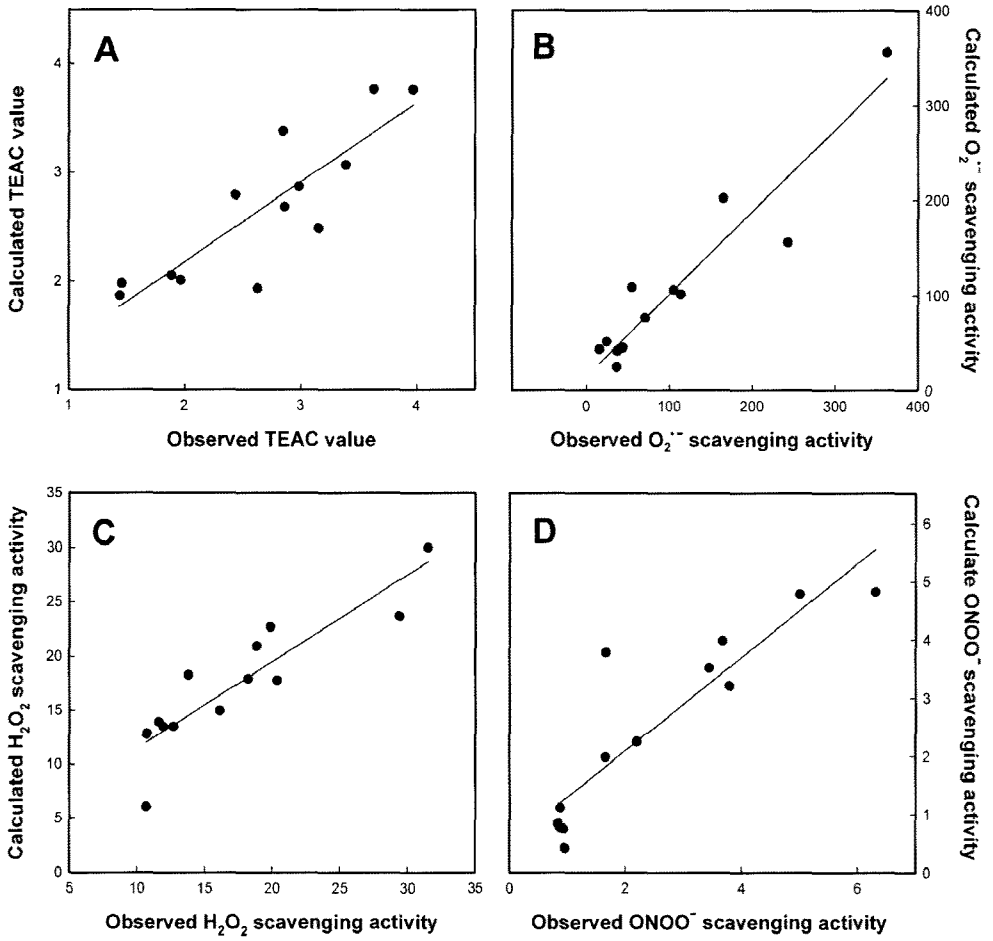


Fig. 1. Plot of observed vs. calculated values of antioxidant activities.

Table 2. Molecular descriptors for anthocyanins included in the best QSAR models[1)]

Compound[2)]	TE	NH	NM	HF	DX	QXY	QYY	QYZ	OZZ
1	1.725	5	0	-13.19	4.221	35.46	42.95	-0.438	-124.74
2	1.706	6	0	-55.96	5.036	19.91	48.29	-4.760	-58.04
3	3.632	4	2	-42.47	6.908	42.38	71.04	-6.067	-138.55
4	0.004	4	0	29.12	2.123	43.45	42.63	0.003	-74.36
5	0.857	4	1	-7.99	4.290	37.70	37.84	0.181	-133.34
6	0.053	5	1	-50.20	7.097	36.49	76.54	-7.808	-137.32
7	16.932	8	0	-245.80	3.914	33.07	-3.31	-1.885	6.71
8	3.748	9	0	-293.38	5.366	42.74	-8.60	2.360	-114.90
9	8.542	7	2	-273.48	2.607	-4.96	83.46	-7.945	-196.79
10	6.520	7	0	-207.55	-3.007	14.12	78.51	5.464	-146.89
11	6.118	7	1	-244.51	-2.938	4.67	53.16	9.201	-205.00
12	2.441	8	1	-286.53	5.604	16.95	10.64	-0.286	-169.15
13	7.144	8	0	-245.80	3.916	33.02	-3.32	-1.576	6.57

[1)]The NH represents the same values as the number of H-bond donors.
[2)]The names and chemical structures of the compounds are presented in Table 1. TE, torsional energy; NH, number of hydroxyl groups; NM, number of methyl groups; HF, heat of formation; DX, dipole X component; QXY, quadrupole XY component; QYY, quadrupole YY component; QYZ, quadrupole YZ component; OZZ, octupole ZZY component.

Table 3. Correlation between antioxidant activities, and descriptors[1)]

	TEAC	O_2^-	H_2O_2	$ONOO^-$	TE	NH	NM	HF	DX	QXY	QYY	QYZ	OZZ
TEAC	1												
$O_2^{\bullet-}$	0.564*	1											
H_2O_2	-0.668*	-0.224	1										
$ONOO^-$	-0.823**	-0.451	0.745**	1									
TE	0.619*	0.833**	-0.150	-0.415	1								
NH	0.828**	0.697**	-0.541	-0.709**	-	1							
NM	-0.254	-0.285	0.550	0.122	-	-	1						
HF	-0.743**	-0.641*	0.322	0.605*	-	-	-	1					
DX	-0.033	-0.051	-0.109	-0.325	-	-	0.180	-	1				
QXY	-0.380	0.056	0.157	0.210	-	-	-0.331	-	0.561*	1			
QYY	-0.533	-0.646*	0.410	0.382	-0.277	-	-	-	-	-	1		
QYZ	0.046	0.159	0.068	0.316	-	0.292	-	-	-	-	-	1	
OZZ	0.365	0.471	-0.364	-0.243	-	-	-	0.084	-	-	-	-	1

[1)]The values represent the correlation coefficient (r) between antioxidant activities (TEAC, $O_2^{\bullet-}$ H_2O_2, and $ONOO^-$ scavenging assays), and descriptors (TE, NH, NM, HF, DX, QXY, QYY, QYZ, and OZZ). TE, torsional energy; NH, number of hydroxyl groups; NM, number of methyl groups; HF, heat of formation; DX, dipole X component; QXY, quadrupole XY component; QYY, quadrupole YY component; QYZ, quadrupole YZ component; OZZ, octupole ZZY component.
*$p<0.05$; **$p<0.01$

The observed values versus the calculated values for the antioxidant activities of anthocyanidins and their glycosides are plotted in Fig. 1. This results demonstrate the high predictive power of the 4 models with good correlations (r^2=0.736, 0.880, 0.802, and 0.802, respectively). Equation 2 had the highest r_2 value (0.880). Generally, the closer the q^2 value to the r^2 value, the higher the predictive power of the model equation. Thus, Eq. 2 had the highest predictive power (q^2=0.811), and this was supported by the result that the r^2 value (0.880) of the plot (observed vs. calculated values, Fig. 1) from Eq. 2 was the highest among the equations.

The correlations between the antioxidant activities and the descriptors are shown in Table 3. The NH showed good correlations with all 4 activities [r=0.828 (p<0.01), 0.697 (p<0.01), −0.541, and −0.709 (p<0.01), respectively]. The correlation between the TEAC value and the $ONOO^-$ scavenging activity had the highest value among the activities (r= −0.823, p<0.01). The TEAC value correlated well with the HF (r= −0.743, p<0.01), and the $O_2^{\bullet-}$ scavenging activity showed a good correlation with the TE (r=0.833, p<0.01).

The 4 QSAR models (Eq. 1-4) for the antioxidant activities of anthocyanidins and their glycosides were derived from multiple linear regression analysis in the current study. This approach requires multicollinearity to be absent, which indicates a high correlation among the dependent variables. In the 4 equations, the correlation coefficients between the descriptors within each equation were very low [r=0.084 (HF vs. OZZ from Eq. 1), 0.277 (TE vs. QYY from Eq. 2), 0.180, 0.561, and −0.331 (NM vs. DX, DX vs. QXY, and NM vs. QXY from Eq. 3), and 0.292 (NH vs. QYZ from Eq. 4), respectively]. These results demonstrated that all of the models followed the principle closely.

Hydrophobicity, topology, steric effects, and electronic parameters are examples of the many descriptors that have been used in QSAR studies across a broad range of disciplines, including drug design and environmental risk assessment (37). The most relevant descriptors of the antioxidant activities of anthocyanidins and their glycosides in the current study were the 3-D structural energy (TE), constitutional (NH and NM), and electrostatic or quantum chemical properties (HF, dipole, quadrupole, and octupole components). Each equation contained one or more electrostatic properties that implied molecular polarizability, which is a measure of the overall electronic-charge distribution that can be distorted by an external electric field and is related to molecular bulk or volume (38,39). The NH correlated well with all 4 assays, and most of the other descriptors (Table 3). This implied that the NH played an important role in antioxidant activities of both anthocyanidins and anthocyanins in our QSAR models. Free hydroxyl groups contribute to antioxidant or free radical-scavenger activities by donating electrons (36). Thus, it was reasonable to count the number of free hydroxyl groups in the sugars to determine the total NH in this study, although it has been reported that sugars in the ring structure can obstruct the antioxidant activities of compounds due to their steric hindrance.

Our QSAR model of anthocyanidins and their glycosides was compared with a QSAR model of the antioxidative activity of flavonoids using a TEAC assay reported by Lien *et al.* (22), as there were no previous reports of QSAR models of the radical-scavenging activities of flavonoids. This approach included 6 aglycones and 3 glycosides (3-glucose, 3-galactose, and 3-rutinose) of anthocyanins as flavonoids, and the TEAC values of all 42 flavonoids extracted from Rice-Evans's results to develop QSAR models (17). Different trends in the TEAC values were noted between the findings of Rice-Evans *et al.* (17) and our group, in that the latter were slightly lower than the former. This phenomenon was considered to be caused by differences in the assay methods used, although the same TEAC assay was carried out.

Another difference between these studies was that Lien *et al.* (22) used only 2 descriptors (the NH and the presence or absence of the 2, 3-double bond in the C ring), and excluded the number of free OH groups in the sugar. In the derived QSAR model, Eq. 1 (TEAC) with r^2=0.736 was obtained in our study, while the corresponding value in the Lien's study was r^2=0.845. However, although the r^2 value in the Lien's model was slightly higher than ours, the predictive power was not comparable, because the former did not present q^2 value. Moreover, the standard deviation (s) in our model (0.451) was slightly lower than that in the Lien's model (0.574). The differences between these 2 QSAR models were considered to result from the different data sets and descriptors included: our model included anthocyanins alone and was governed by electrostatic properties, while Lien's model included various flavonoids and was governed by the NH and the presence or absence of 2, 3-double bond in the C ring.

In summary, our study developed 4 reliable QSAR equations with significant descriptors, 3-D structural energy, constitutional, and electrostatic parameters, by multiple linear regression analysis. To our knowledge, the first report of QSAR models for the antioxidant activities of anthocyanins alone. The derived QSAR models should provide valuable guidelines as to the physicochemical descriptors that influence the antioxidant activities of anthocyanins, and might also be helpful in predicting the activities of other anthocyanins.

Acknowledgments

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