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Characterization of Oligomeric Procyanidins and Identification of Quercetin Glucuronide from Lotus (*Nelumbo nucifera* Gaertn.) Seedpod

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Supporting Information

ABSTRACT: Procyanidins are a class of polyphenols in the plant kingdom. Lotus (*Nelumbo nucifera* Gaertn.) seedpods, the inedible part of lotus and a byproduct during the production of lotus seeds, were found to be a new source rich in procyanidins. Detailed information about oligomeric procyanidins in lotus seedpods remains unknown. In this study, lotus seedpods were extracted using 60% aqueous methanol and characterized with phloroglucinolysis and liquid chromatography (mass spectrometry with an electrospray ionization source). The results indicate that the oligomeric and polymeric fraction had a mean degree of polymerization of 3.2 and 15.4, respectively, and consisted of (+)-catechin (m/z 289), gallocatechin or epigallocatechin (m/z 305), quercetin glycoside (m/z 463), quercetin glucuronide (m/z 477), procyanidin dimers (m/z 577.1), proanthocyanidin dimer gallate (m/z 593.3), prodelphinidin dimers (m/z 609.1), procyanidin trimers (m/z 865.1), etc. Quercetin glucuronide was further purified using flash chromatography and identified as quercetin-3-*O*-β-glucuronide by determining its exact mass using ion-trap time-of-flight mass spectrometry and ^{1}H and ^{13}C nuclear magnetic resonance, ^{1}H-detected heteronuclear single-quantum coherence, and ^{1}H-detected heteronuclear multiple-bond correlation analyses.

KEYWORDS: *lotus seedpod, procyanidins, gallocatechin, quercetin-3-O-β-glucuronide, exact mass, HSQC, HMBC*

INTRODUCTION

Procyanidins, also known as condensed tannins, are a class of polyphenols common in the plant kingdom. They are composed of chains of flavan-3-ol units [i.e., (+)-catechin and (−)-epicatechin] linked mainly through C4−C8 or C4−C6 bonds.[1] Some procyanidins may be esterified to gallic acid (Figure 1). Procyanidins from grape seeds, French maritime pine bark, cocoa beans, cranberries, and apples have been extensively studied.[1−6] They have documented health benefits, including cardiovascular protection,[7] low-density lipoprotein oxidation inhibition,[8] anti-inflammation,[9] and antitumor-proliferation properties.[10]

Lotus (*Nelumbo nucifera* Gaertn.) seedpods, the inedible part of the lotus, are rich in oligomeric procyanidins[11] and could be a new source of procyanidins. However, detailed information about procyanidins in the lotus seedpod is lacking. In this study, procyanidins were extracted from the lotus seedpod and characterized by liquid chromatography−mass spectrometry. During the study, a compound with a large abundance, referred to in this study as compound **1**, became of interest and was identified by ^{1}H-detected heteronuclear single-quantum coherence (HSQC) and ^{1}H-detected heteronuclear multiple-bond correlation (HMBC) analyses and accurate mass determination with ion-trap time-of-flight tandem mass spectrometry (IT-TOF-MS). Exact mass determination using IT-TOF-MS can differentiate compounds or molecular fragments with different elemental compositions and similar normal-resolution mass and function well in structure elucidation and fragmentation pathway deduction. The HSQC and HMBC analyses allow for the complete elucidation of the compound structure, including the glycosidation site and steric conformation of the chemical bond.

MATERIALS AND METHODS

Chemicals and Reagents. Acetonitrile (Fisher, Waltham, MA) was high-performance liquid chromatography (HPLC)-grade. (+)-Catechin, (−)-epicatechin, (+)-gallocatechin, (−)-epigallocatechin, phloroglucinol, and dimethyl sulfoxide-d_6 (Sigma, St. Louis, MO) were HPLC-grade. A standard mixture of procyanidins B1, B2, B3, and C1 was prepared from 'Granny Smith' apples according to the method described previously.[12] Other chemicals and reagents were of analytical grade, unless specified.

Isolation of Procyanidins from Lotus Seedpods. Lotus seedpods were collected from Tang Xun Lake in July 2006 (Wuhan, China) and stored at −20 °C until use. After the seeds were dissected, 100 g of fresh lotus seedpods were torn into small pieces by hand and extracted twice in a flask with 60% (v/v) aqueous methanol (200 mL) at 50 °C for 2 h. The extracts were combined and filtered under reduced pressure, and the filtrate was concentrated by rotatory evaporation at 40 °C under reduced pressure until the methanol was removed. The residue was applied to a 300 × 30 mm inner diameter column packed with AB-8 macroporous resin (Nankai Hecheng, Tianjing, China). The column was rinsed with 2500 mL of distilled water and eluted with 100 mL of 70% aqueous ethanol (v/v) at a flow rate of 10 mL/min. The eluent was concentrated by rotatory

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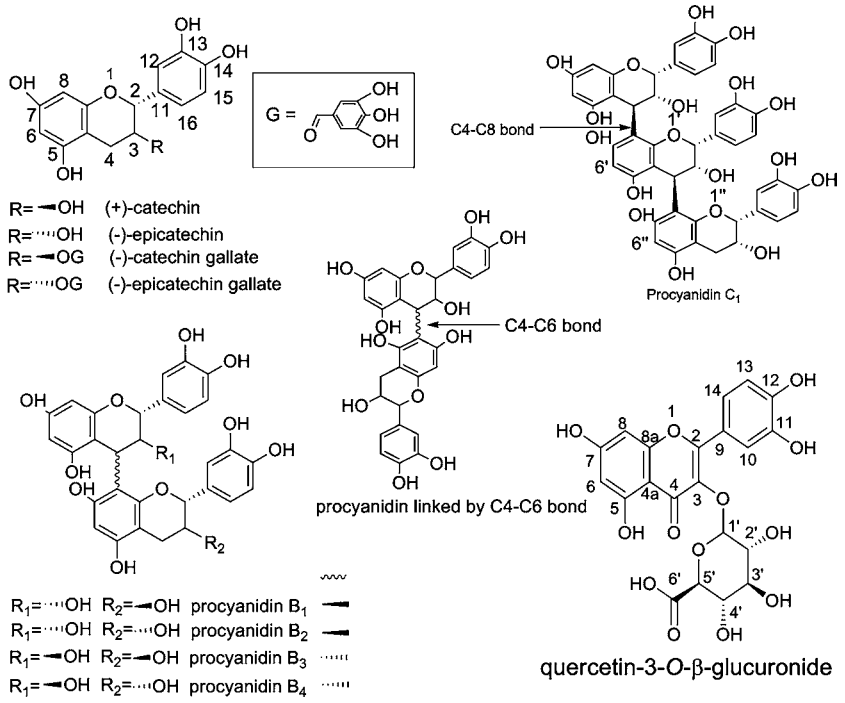

Figure 1. Chemical structure of procyanidins and quercetin-3-*O*-*β*-glucuronide.

evaporation at 40 °C under reduced pressure to remove the ethanol and then freeze-dried. At the conclusion of this process, lotus procyanidin extract (LPE), a brown powder, was obtained.

LPE was dissolved in deionized water and extracted twice, each time with an equal volume of ethyl acetate. The ethyl acetate and water phase were concentrated by rotatory evaporation under reduced pressure at 40 °C and freeze-dried, and two lotus seedpod procyanidin fractions, specifically LPE1 and LPE2, were obtained.

Liquid Chromatography–Electrospray Ionization–Mass Spectrometry (LC–ESI–MS) Analysis of LPE1. LPE1 was analyzed on an Agilent 1100 LC–ESI–MS system (Agilent, Foster City, CA). The HPLC analyses were run according to the following conditions. The column used was a 150 × 2.1 mm inner diameter, 5 μm, Zorbax SB-C18, with a 4 × 4 mm inner diameter guard column of the same material (Agilent Technologies, Palo Alto, CA). The mobile phase consisted of 0.2% aqueous acetic acid as A and acetonitrile as B with the following gradient: from 5 to 15% B in the first 10 min, from 15 to 20% B in the next 5 min, from 20 to 40% B in the next 20 min, from 40 to 50% B in the next 10 min, from 50 to 5% B in the next 5 min, and re-equilibrated with 5% B for 5 min before the next injection. The flow rate was 0.2 mL/min and monitored at 280 nm with a diode array detector. Mass spectra were collected using an ESI ion source in the negative mode under the following conditions: fragmenter voltage, 100 V; capillary voltage, 2500 V; nebulizing pressure, 30 psi; dry gas temperature, 300 °C; and mass range, m/z 100–2200.

Preparation of Compound 1. During the LC–ESI–MS analysis of LPE1, compound **1** (m/z 477.2) was discovered (Figure 1). Compound **1** was isolated from LPE using the following process. A total of 500 g of LPE was suspended in acetic-acid-acidified acetone, and the precipitate was collected by centrifugation, dissolved in water, and applied to a column packed with 200 mesh polyamide resin (Mosu Scientific Instrument Company, Shanghai, China). The column was eluted with 95, 80, 60, and 40% (v/v) aqueous ethanol in sequence. The 40% eluent was combined and freeze-dried to obtain the compound **1** crude extract. It was further purified by a flash chromatography system (Lisui Co., Ltd., Shanghai, China). The crude extract was dissolved in 5% (v/v) aqueous methanol and applied to a column of 400 × 40 mm inner diameter (Buchi, Flawil, Switzerland) packed with C18 resin (particle size of 30–50 μm). The elution gradient using aqueous methanol was as follows: 20% methanol at the beginning, a linear increase to 45% methanol after 20 min, followed by 45% methanol for 25 min. Fractions from 33 to 38 min were collected and freeze-dried, and a final weight of 4.5 g of compound **1** was obtained.

Identification of Compound 1. Structurally informative fragments and their exact mass within compound **1** were obtained on a Shimadzu tandem ion-trap time-of-flight mass spectrometer equipped with an ESI interface (Shimadzu, Kyoto, Japan). The analysis was conducted in the negative mode under the following operating parameters: probe voltage, 4.5 kV; nebulizer nitrogen gas, 1.5 L/min; curved desolvation line temperature, 200 °C; ion trapping time, 30 ms; collision-induced dissociation energy, 50%; collision gas, 50%; collision-induced dissociation time, 50 ms.

For further identification of compound **1**, ^{1}H and ^{13}C nuclear magnetic resonance (NMR), HSQC, and HMBC spectroscopies were performed on a 400 MHz Bruker Avance II (Bruker Biospin, Rheinstetten, Germany) at room temperature in dimethyl sulfoxide-d_6. The assignments of these spectra were summarized in Table 1.

RESULTS AND DISCUSSION

Characterization of LPE1 and LPE2. The mean degree of polymerization and formation of terminal and extension units of LPE1 and LPE2 were determined by phloroglucinolysis (Table 2) according to the method described previously.[13] The mean degree of polymerization of LPE1 and LPE2 was 3.2 and 15.4, respectively. This indicates that the ethyl acetate phase of LPE contains mainly oligomeric procyanidins, while the water phase contains the polymeric procyanidins. Both LPE1 and LPE2 contained catechin and epicatechin as terminal units and catechin, epicatechin, and epigallocatechin as extension units. The epicatechin units accounted for 74 and 93% of the terminal units in LPE1 and LPE2, respectively. The epigallocatechin unit proportion was high in extension units of LPE1 (31%) and LPE2 (54%), and their constituent unit profile was quite different from that in apple procyanidins[12] (mainly catechin or epicatechin units) and grape seed procyanidins[3] (mainly catechin or catechin gallate as constituent units).

In a HPLC chromatogram of LPE1 (Figure 2A), peaks 1, 2, and 3 gave the maximum absorption at 279 nm and

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Table 1. ^{13}C NMR, HSQC, and HMBC Peak Assignments of Quercetin-3-*O*-*β*-glucuronide

carbon number	δ_C	HSQC (δ_H)	HMBC
2	156.66		H-10, H-14
3	133.50		H-1′
4	177.62		
4a	104.31		H-6, H-8
5	161.63		H-6
6	99.23	6.19 d	H-8
7	164.69		H-6, H-8
8	94.05	6.39 d	H-6
8a	156.71		H-8
9	121.8		
10	116.49	7.53 br s	
11	145.38		H-10, H-13
12	149.08		H-10, H-13, H-14
13	115.65	6.8 d	H-14
14	122.15	7.58 dd	H-10, H-13
1′	101.50	5.49 d	H-2′, H-3′
2′	74.22	3.2−3.4[a]	*b*
3′	76.28	3.2−3.4[a]	*b*
4′	71.81	3.2−3.4[a]	*b*
5′	76.41	3.56 d	H-3′, H-4′
6′	170.32		H-4′, H-5′

[a]These protons could not be assigned accurately because of the interference of H_2O. [b]Although long-range correlations are observed, they could not be resolved because of the failure to assign related protons.

Table 2. Compositional Unit Profile of LPE1 and LPE2

	LPE1		LPE2	
constituent units	terminal unit (mol %)	extension unit (mol %)	terminal unit (mol %)	extension unit (mol %)
(+)-catechin	74	26	93	13
(−)-epicatechin	26	43	7	33
epigallocatechin	not detected	31	not detected	54
mean degree of polymerization	3.2		15.4	

corresponded to extracted ion current chromatogram (EIC) peaks of *m*/*z* 577, 289, and 865. This suggested that peaks 1, 2, and 3 were procyanidin dimers, monomers (i.e., catechin or epicatechin), and trimers. By co-elution with procyanidins B1, B2, and B3, peak 1 was confirmed as procyanidin B3. From its ESI−MS spectrum (Figure 2B) injected by a syringe pump, the compounds in LPE1 could be tentatively identified according to their *m*/*z* ratios as follows: catechin or epicatechin (*m*/*z* 289), gallocatechin or epigallocatechin (*m*/*z* 305), quercetin glycoside (*m*/*z* 463), quercetin glucuronide (*m*/*z* 477), procyanidin dimers (*m*/*z* 577.1), proanthocyanidin dimer gallate (*m*/*z* 593.3), prodelphinidin dimers (*m*/*z* 609.1), procyanidin trimers (*m*/*z* 865.1), proanthocyanidin trimers with one gallocatechin or epigallocatechin unit and two catechin units (*m*/*z* 881.4), proanthocyanidin trimers with two gallocatechin or epigallocatechin units and one catechin or epicatechin unit (*m*/*z* 897.3), and procyanidin tetramers (*m*/*z* 1153.1). Proanthocyanidin monomers, dimers, trimers, and tetramers were found in LPE1, which indicated that lotus seedpod, a byproduct of lotus seed production, could be another rich source of natural proanthocyanidins in addition to grape seeds, apples, and French maritime pine bark. Moreover, lotus seedpods could be a unique source of procyanidins consisting of catechin and gallocatechin units as a result of their different compositions and bioactivities compared to other procyanidin extracts, because the bioactivities of procyanidins are closely related to their structures.

Identification of Compound 1. The exact mass of compound **1** and its product ions in MS were determined with an ion-trap time-of-flight mass spectrometer using an ESI ion source in the negative mode. An exact mass measurement was capable of differentiating compounds of varying elemental composition but similar molecular mass at normal mass resolution and could be used in structure and fragmentation elucidation studies.[14] The parent ion and product ions and their calculated *m*/*z*, measured *m*/*z*, and mass error between the calculated and detected values were summarized in Table 3. The low-resolution MS of compound **1** indicated many possible molecular formula, including $C_{22}H_{22}O_{12}$ and $C_{21}H_{18}O_{13}$, but exact mass determination by IT-TOF-MS indicated an elemental composition of $C_{21}H_{18}O_{13}$, with a mass

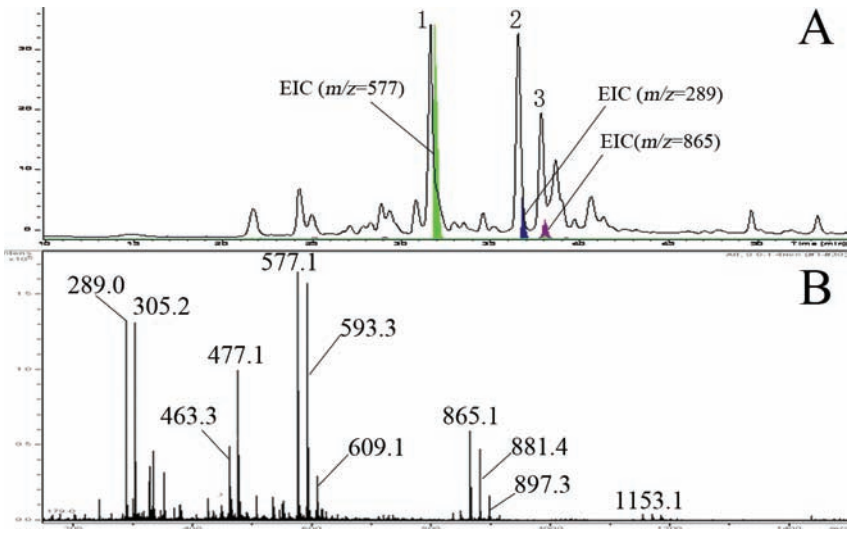

Figure 2. HPLC chromatogram, EICs, and mass spectrum of LPE1 using LC−ESI−MS.

Table 3. Exact Mass Measurement of Quercetin-3-*O*-*β*-glucuronide Fragments Using IT-TOF-MS in ESI Negative Mode

fragment	formula	calculated mass	measured mass	mass error (mDa)
compound **1**	$C_{21}H_{17}O_{13}$	477.0675	477.0678	0.3
1	$C_{15}H_9O_7$	301.0354	301.0334	2
2	$C_8H_3O_5$	178.9986	178.9984	0.2
3	$C_7H_3O_4$	151.0037	151.0058	2.1

error of 0.3 mDa. The MS^2 spectrum indicated the existence of the product ion of $C_{15}H_{10}O_7$. The MS^3 spectrum indicated the existence of product ions of $C_8H_3O_5$ and $C_7H_3O_4$. The possible fragmentation pathway was elucidated in Figure 3 and corresponded to a previous report.[15]

In the ^1H NMR spectrum, all linking-to-carbon protons were assigned, except three protons on C2′, C3′, and C4′, because of the interference of the H_2O/HDO peak. Five total sp^2 carbon proton (δ 6.19−7.58) signals were observed, two of which (δ 6.19 and 6.39) were assigned to C6 and C8 of the A ring of the quercetin moiety and three of which (δ 7.53, 6.8, and 7.58) were assigned to C10, C13, and C14 of the B ring of the quercetin moiety. The coupling constant of the anomeric proton (H1′, δ 5.49, $J_{H1'-H2'}$ = 7.53 Hz) suggested a configuration of the glycoside bonds. The ^{13}C NMR analysis of compound **1** assigned six carbon signals to a glucuronic acid moiety, including one anomeric carbon (δ 101.50) and one carboxyl carbon (δ 170.32), while the other carbons were assigned to an aglycone moiety. Additional carbons were also assigned (Table 1) and were consistent with the quercetin structure. Peaks in the HSQC spectrum occurred at C6, C8, C10, C13, C14, C1′, and C5′ and were coupled to the corresponding protons (Table 1), which supported the theory that compound **1** could be quercetin glucuronide. The glycosidation site was revealed to be C3 of the quercetin moiety by the HMBC experiment, which indicated a long-range correlation between C3 (δ 133.50) and the anomeric proton (δ 5.49). Other long-range correlations between C2 and H10 and H14, C7 and H6 and H8, and C12 and H10, H13, and H14 were observed from the HMBC spectrum (Table 1) and were in good agreement with quercetin-3-*O*-*β*-glucunoride. All NMR spectra suggested that compound **1** is quercetin-3-*O*-*β*-glucunoride and are consistent with the study by Moon et al.[16] However, because of assignment failure of H2′, H3′, and H-4′, the related peaks in the HSQC and HMBC spectra were not thoroughly assigned.

Quercetin-3-*O*-*β*-glucuronide is considered a metabolite of quercetin[16−18] and acts as a low-density lipoprotein oxidation inhibitor, 1,1-diphenyl-2-picryhydrazyl radical scavenger,[16] angiotensin-II-induced vascular smooth muscle cell hypertrophy inhibitor,[19] cell migration and proliferation inhibitor,[20] and lung cancer cell growth inhibitor.[21] Human *β*-glucuronidase, sulfotransferase, catechol-*O*-methyltransferase, and multi-resistant protein 2 were involved in its *in vivo* synthesis,[18] and deglucuronidation was involved in its functioning.[22] Quercetin-3-*O*-*β*-glucuronide was also found in other plants (lotus leaves[23] and fennel[24]). Lotus seedpod could be another source of quercetin-3-*O*-*β*-glucuronide.

In conclusion, detailed information about the procyanidins in lotus seedpod was obtained. Procyanidins from lotus seedpod had a high content of gallocatechin or epigallocatechin units as their constituent units, which was different from procyanidins from grape seeds, apples, or pine bark. The molecular formula and fragmentation pathway of quercetin-3-*O*-*β*-glucuronide were obtained by determining the exact mass, and its structure was also verified by employing ^1H and ^{13}C NMR, HSQC, and HMBC analyses.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

^1H and ^{13}C NMR, HSQC, and HMBC spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

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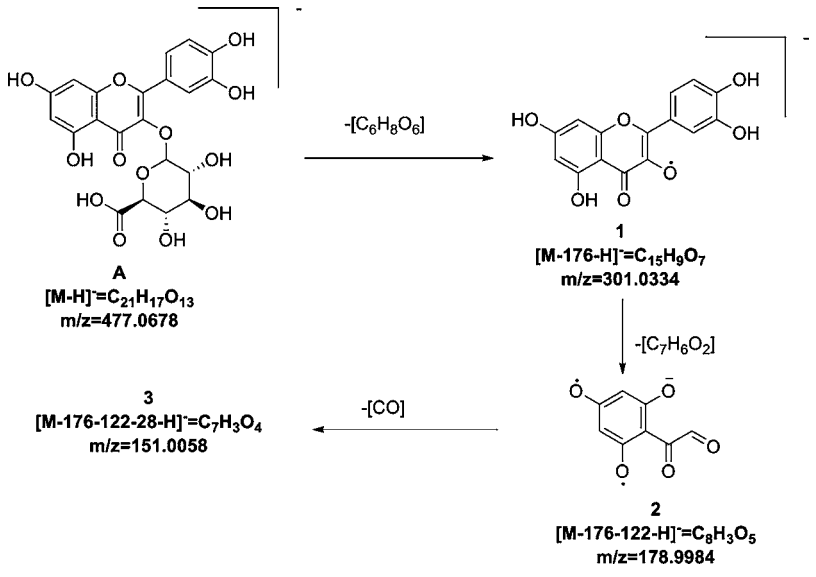

Figure 3. Possible fragmentation pathway of quercetin-3-*O*-*β*-glucuronide deduced using IT-TOF-MS in ESI negative mode.

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Notes

The authors declare no competing financial interest.

■ ABBREVIATIONS USED

LPE, lotus procyanidin extract; LC−ESI−MS, liquid chromatography−electrospray ionization−mass spectrometry; IT-TOF-MS, ion-trap time-of-flight mass spectrometry; HSQC, ^{1}H-detected heteronuclear single-quantum coherence; HMBC, ^{1}H-detected heteronuclear multiple-bond correlation; NMR, nuclear magnetic resonance

■ REFERENCES

(1) Lazarus, S. A.; Adamson, G. E.; Hammerstone, J. F.; Schmitz, H. H. High-performance liquid chromatography/mass spectrometry analysis of proanthocyanidins in foods and beverages. *J. Agric. Food Chem.* **1999**, *47*, 3693−3701.

(2) Rohdewald, P. A review of the French maritime pine bark extract (Pycnogenol), a herbal medication with a diverse clinical pharmacology. *Int. J. Clin. Pharmacol. Ther.* **2002**, *40*, 158−168.

(3) Prieur, C.; Rigaud, J.; Cheynier, V.; Moutounet, M. Oligomeric and polymeric procyanidins from grape seeds. *Phytochemistry* **1994**, *36*, 781−784.

(4) Adamson, G. E.; Lazarus, S. A.; Mitchell, A. E.; Prior, R. L.; Cao, G.; Jacobs, P. H.; Kremers, B. G.; Hammerstone, J. F.; Rucker, R. B.; Ritter, K. A.; Schmitz, H. H. HPLC method for the quantification of procyanidins in cocoa and chocolate samples and correlation to total antioxidant capacity. *J. Agric. Food Chem.* **1999**, *47*, 4184−4188.

(5) Lu, Y.; Yeap Foo, L. Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chem.* **2000**, *68*, 81−85.

(6) de Freitas, V. A. P.; Glories, Y.; Bourgeois, G.; Vitry, C. Characterisation of oligomeric and polymeric procyanidins from grape seeds by liquid secondary ion mass spectrometry. *Phytochemistry* **1998**, *49*, 1435−1441.

(7) Keen, C. L.; Holt, R. R.; Oteiza, P. I.; Fraga, C. G.; Schmitz, H. H. Cocoa antioxidants and cardiovascular health. *Am. J. Clin. Nutr.* **2005**, *81*, 298s−303s.

(8) Pearson, D. A.; Schmitz, H. H.; Lazarus, S. A.; Keen, C. L.; Lester, P. Inhibition of *in vitro* low-density lipoprotein oxidation by oligomeric procyanidins present in chocolate and cocoas. *Methods Enzymol.* **2001**, *335*, 350−360.

(9) Schramm, D.; Wang, J.; Holt, R. Chocolate procyanidins decrease the leukotriene−prostacyclin ratio in humans and human aortic endothelial cells. *Am. J. Clin. Nutr.* **2001**, *73*, 36−40.

(10) Zhao, J.; Wang, J.; Chen, Y.; Agarwal, R. Anti-tumor-promoting activity of a polyphenolic fraction isolated from grape seeds in the mouse skin two-stage initiation−promotion protocol and identification of procyanidin B5−3′-gallate as the most effective antioxidant constituent. *Carcinogenesis* **1999**, *20*, 1737−1745.

(11) Ling, Z. Q.; Xie, B. J.; Yang, E. L. Isolation, characterization, and determination of antioxidative activity of oligomeric procyanidins from the seedpod of *Nelumbo nucifera* Gaertn. *J. Agric. Food Chem.* **2005**, *53*, 2441−2445.

(12) Xiao, J. S.; Liu, L.; Wu, H.; Xie, B. J.; Yang, E. N.; Sun, Z. D. Rapid preparation of procyanidins B2 and C1 from Granny Smith apples by using low pressure column chromatography and identification of their oligomeric procyanidins. *J. Agric. Food Chem.* **2008**, *56*, 2096−2101.

(13) Kennedy, J. A.; Jones, G. P. Analysis of proanthocyanidin cleavage products following acid-catalysis in the presence of excess phloroglucinol. *J. Agric. Food Chem.* **2001**, *49*, 1740−1746.

(14) Hopfgartner, G.; Chernushevich, I. V.; Covey, T.; Plomley, J. B.; Bonner, R. Exact mass measurement of product ions for the structural elucidation of drug metabolites with a tandem quadrupole orthogonal-acceleration time-of-flight mass spectrometer. *J. Am. Soc. Mass Spectrom.* **1999**, *10*, 1305−1314.

(15) Tiberti, L. A.; Yariwake, J. H.; Ndjoko, K.; Hostettmann, K. On-line LC/UV/MS analysis of flavonols in the three apple varieties most widely cultivated in Brazil. *J. Braz. Chem. Soc.* **2007**, *18* (1), 100−105.

(16) Moon, J.-H.; Tsushida, T.; Nakahara, K.; Terao, J. Identification of quercetin 3-*O*-β-D-glucuronide as an antioxidative metabolite in rat plasma after oral administration of quercetin. *Free Radical Biol. Med.* **2001**, *30*, 1274−1285.

(17) Wittig, J.; Herderich, M.; Graefe, E. U.; Veit, M. Identification of quercetin glucuronides in human plasma by high-performance liquid chromatography−tandem mass spectrometry. *J. Chromatogr., B: Biomed. Sci. Appl.* **2001**, *753*, 237−243.

(18) O'Leary, K. A.; Day, A. J.; Needs, P. W.; Mellon, F. A.; O'Brien, N. M.; Williamson, G. Metabolism of quercetin-7- and quercetin-3-glucuronides by an in vitro hepatic model: The role of human β-glucuronidase, sulfotransferase, catechol-*O*-methyltransferase and multi-resistant protein 2 (MRP2) in flavonoid metabolism. *Biochem. Pharmacol.* **2003**, *65*, 479−491.

(19) Yoshizumi, M.; Tsuchiya, K.; Suzaki, Y.; Kirima, K.; Kyaw, M.; Moon, J.-H.; Terao, J.; Tamaki, T. Quercetin glucuronide prevents VSMC hypertrophy by angiotensin II via the inhibition of JNK and AP-1 signaling pathway. *Biochem. Biophys. Res. Commun.* **2002**, *293*, 1458−1465.

(20) Ishizawa, K.; Izawa-Ishizawa, Y.; Ohnishi, S.; Motobayashi, Y.; Kawazoe, K.; Hamano, S.; Tsuchiya, K.; Tomita, S.; Minakuchi, K.; Tamaki, T. Quercetin glucuronide inhibits cell migration and proliferation by platelet-derived growth factor in vascular smooth muscle cells. *J. Pharmacol. Sci.* **2009**, *109*, 257−264.

(21) Yang, J. H.; Hsia, T. C.; Kuo, H. M.; Chao, P. D. L.; Chou, C. C.; Wei, Y. H.; Chung, J. G. Inhibition of lung cancer cell growth by quercetin glucuronides via G_2/M arrest and induction of apoptosis. *Drug Metab. Dispos.* **2006**, *34*, 296−304.

(22) Lee-Hilz, Y. Y.; Stolaki, M.; van Berkel, W. J. H.; Aarts, J. M. M. J. G.; Rietjens, I. M. C. M. Activation of EpRE-mediated gene transcription by quercetin glucuronides depends on their deconjugation. *Food. Chem. Toxicol.* **2008**, *46*, 2128−2134.

(23) Lin, H.-Y.; Kuo, Y.-H.; Lin, Y.-L.; Chiang, W. Antioxidative effect and active components from leaves of lotus (*Nelumbo nucifera*). *J. Agric. Food Chem.* **2009**, *57*, 6623−6629.

(24) Parejo, I.; Viladomat, F.; Bastida, J.; Schmeda-Hirschmann, G.; Burillo, J.; Codina, C. Bioguided isolation and identification of the nonvolatile antioxidant compounds from fennel (*Foeniculum vulgare* Mill.) waste. *J. Agric. Food Chem.* **2004**, *52*, 1890−1897.